NEUROBEHAVIORAL QUANTIFICATION OF THE TRANSITION TO EXPLICIT AWARENESS IN SKILLED MOTOR LEARNING: IMPLICATIONS FOR REHABILITATION

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NEUROBEHAVIORAL QUANTIFICATION OF THE TRANSITION TO EXPLICIT AWARENESS IN SKILLED MOTOR LEARNING: IMPLICATIONS FOR REHABILITATION

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Success is a journey, not a destination. The doing is often more important than the outcome.

Arthur Ashe

For my parents.

This dissertation is dedicated to my parents, Barbara and Pat, who are the rock upon which I stand. They are two of the most amazing people I know, full of kindness, generosity, love and life. They have always been there providing support and encouragement. Celebrating the successes, providing a shoulder through the challenges, and helping me to power through obstacles. Their unwavering belief gave me the confidence and courage to pursue my dreams, making this accomplishment a reality. For all that you are, and all that you have given me, I thank you. Love you always.

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SUMMARY

We often take for granted the ability to execute sequential movements in a smooth, automatic manner, whether parallel parking, tying a shoe or making the winning catch in the World Series. Learning these skills requires the ability to concatenate a sequential series of individual movement patterns into a fluid motion. Unfortunately, many patient populations exhibit deficits in motor learning, impairing the ability to develop such sequential motor skills. Most notably, previous studies in our lab have demonstrated impaired motor learning for prosthesis users, resulting in device abandonment issues. Understanding the individual neural progression associated with sequential learning in healthy individuals may provide valuable insights of motor learning as well as identify factors that impede learning.

At the start of the motor learning process, movements are often slow and jerky, requiring a concerted focused effort. With practice, movement execution becomes more fluid, requiring less focused attention and skills that are transferable to similar, but novel situations. Prior studies of motor learning have demonstrated both beneficial and detrimental effects of practice to a level of explicit awareness. Such conflicting results may be attributed to the differences in protocol for the experiments. Specifically, it was noted that beneficial effects such as improved perceptual sensitivity and enhanced movement vigor, were found for awareness that developed incidentally, suggesting a potential therapeutic benefit. However, previous studies of incidental awareness are limited by the use of a group analysis methodology, not addressing the individual variability in motor learning. This method potentially masks vital transient changes which may provide additional insight into the neural basis of motor skill learning, suggesting a need for an individualized approach. Therefore, the overall goal of this dissertation was to identify an individualized-indicator of incidentally developed explicit awareness. This indicator allows a more precise examination of neurobehavioral changes associated with, and effects of, sequential motor learning to a level of explicit awareness. Neurobehavioral changes were examined for healthy, intact individuals in Aims 1 and 2, with Aim 3 also including prosthesis users.

Aim 1 provided a reliable indirect indicator for both the presence and timing of explicit awareness development. Utilization of the indicator, along with electroencephalography (EEG), revealed the presence of a facilitative frontoparietal network recruited for subjects who developed awareness. Aim 2 results provided additional validation of the individualized indicator, revealing the impact of both working memory and learning strategy on the ability to transfer recently-learned motor skills to a novel, more complex task. Aim 3 utilized a three-dimensional reach-and-grasp task to examine visual and motor contributions to motor learning separately. Prosthesis users demonstrated neurobehavioral patterns reflective of enhanced visual reliance for motor control, thus impacting motor learning progression. Prosthesis users developing awareness appeared to engage in behaviors which introduced additional sensorimotor information relevant to motor learning. The individualized approach in the presented dissertation may provide insight for individualized rehabilitative approaches to assist patients experiencing motor learning deficits.

CHAPTER 1
INTRODUCTION AND BACKGROUND

1.1 Problem Definition

Sequential motor tasks are ubiquitous in our daily lives. Whether a professional athlete making the winning catch in the World Series, a child tying his shoes, or an adult parallel parking into a tight spot, we all rely on sequences of motor movements to accomplish our goals. Learning the elements that build to these sequential motor skills requires a complex interaction between multiple brain regions to produce the fluid, automatic motions that we have all come to expect. Unfortunately, multiple populations and disease states have demonstrated sequential motor learning deficits including aging, stroke, multiple sclerosis, autism and amputation [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Understanding the neural changes associated during sequential motor learning in healthy individuals may provide insights into potential interventions in these populations.

At the start of this motor learning process, initial movements are often slow and jerky, requiring concerted effort [13, 14]. With repeated practice, however, improvements in motor execution often result in faster, more fluid movements requiring less conscious focus and are transferable to similar, but novel situations [15, 16, 17, 18, 19]. Multiple studies have provided evidence that there are two parallel processing systems involved in the acquisition and execution of such skills, the implicit and explicit systems [20, 21, 22]. The implicit system is proposed to be involved in optimizing movement execution, while the explicit system is primarily involved in goal selection and execution. Prior sequential motor learning studies have provided valuable insight into the neurobehavioral correlates of each system associated with motor skill acquisition. However, conflicting studies indicate explicit awareness can be detrimental [23, 24, 25, 26, 27] or beneficial [28, 29, 30, 31] in the acquisition and generalization of motor skills. One attribute which may contribute to the observed conflicting results is the experimental method utilized by the subject for aware-

ness development, intentional instruction or incidental discovery. Examining the impact of explicit awareness on motor skill acquisition requires the ability to know that a subject has awareness of their movement patterns. While knowing if a subject has awareness at the end of an experimental session can easily be assessed with recall methods, examining the neurobehavioral changes occurring during the development of awareness presents the challenge of knowing when awareness develops due to variability in learning rates.

Currently, most motor learning studies examine changes associated with motor learning from a group perspective, potentially missing vital, transient, changes that occur during an individuals learning progression. When performing group averaging on behavioral or neural changes occurring during learning, the significance of individual variability may be artificially raised, lowered, or muted in statistical power, resulting in misleading conclusions or misinterpreted data. The significance of these differences may help explain motor skill training rehabilitative interventions which report the presence of responders and non-responders [32, 33, 34, 35]. This implies that an individuals neural state during the intervention may facilitate or impede responsivity to the intervention.

Therefore, the current study aims to identify behavioral predictors of individualized learning rate in a sequential motor task in which explicit awareness develops incidentally. Such a predictor will provide a tool with which to examine the oculomotor, motor, and neural changes correlative with progression or impediments in motor skill acquisition. Correlations noted to be present for relevant behavioral changes can introduce novel rehabilitative measures with which to provide more efficient and effective individualized interventions and instructions.

1.2 Background

1.2.1 Motor Skill Learning

Successful motor skill learning typically involves repetitive practice resulting in the production of movements which are executed quickly, smoothly, accurately and effortlessly, without conscious thought, and are transferable to similar, but novel situations. Examples of these motor skills are easily found in daily life, such as playing the piano, catching a baseball, driving a car, or grasping

and manipulating small objects. Studying the acquisition of motor skills in experimental settings, however, typically utilize paradigms falling into two major categories: (1) the concatenation of individual movements into a fluidly executed motor behavior, known as sequential motor learning, and (2) the motor compensation observed in response to an environmental perturbation, known as motor adaptation. The current study focuses on the neurobehavioral changes associated with sequential motor learning.

Studies have shown that motor skill learning occurs in two distinct stages, with transition between these two stages being assisted by an intermediate stage [36, 37, 38, 39, 19]. The first stage, fast learning, demonstrates rapid improvements in movement execution within a single session, with observable changes occurring on a time scale of minutes. These improvements are typically seen as movements executed more quickly and accurately [36]. The second stage, slow learning, appears to involve optimization of movement patterns resulting in faster, more fluid movements, requiring extensive practice of months or even years [40, 41]. The intermediate stage, involves consolidative processes, resulting in skill stabilization of fast learning motor representations and performance improvements in the absence of practice, providing a connection between the fast and slow learning stages [42, 43].

1.2.2 Neural correlates of motor learning stages

The first, fast stage of motor learning is known to show activity in multiple brain regions including the dorsolateral prefrontal cortex (dlPFC), posterior parietal regions (PPC), primary motor cortex (M1), supplementary motor area (SMA), basal ganglia striatal regions (BG) and cerebellum (CB) [36, 44]. The aforementioned regions make up two parallel loop circuits utilized during the first stage of learning that involves the convergence of spatial and motor coordination [45]. Spatial coordination involves fronto-parietal associations (dlPFC-PPC) with the BG-CB circuit, in which increased frontoparietal activations are correlated with performance gains typical of the first stage [45]. Motor coordination involves the M1-SMA-BG-CB circuit whose activation decreases with learning, suggestive of increased efficiency [46, 47]. Transformation between spatial and motor

features is a necessary determinant of fast motor learning and relies on the SMA to shift fronto-parietal associations to the sensorimotor-striatal circuit [45].

The second, slow stage of motor learning, involves extensive practice which is suggested to provide synaptic enhancement of cortico-striatal and cortico-cerebellar connections and disengagement of fronto-parietal circuits [36, 45]. The fronto-parietal disengagement allows the sensorimotor-striatal circuit to execute movements, seen as automatic motor execution, no longer requiring conscious focus [36, 48].

The transition between fast and slow learning involves the intermediate stage, which has been shown to engage a sensorimotor-striatal circuit providing the substrate for the acquisition of habitual and automatic behaviors via consolidation. Consolidation processes are thought to involve improvement in synaptic strength through utilization of long-term potentiation (LTP) and long-term depression (LTD) like mechanisms [49, 50, 51, 52, 53]. While many studies have demonstrated the offline performance gains seen with consolidation are most apparent after sleep, recent studies have also shown that the benefits of consolidation resulting in skill stabilization can be observed without sleep and are associated with the presence of explicit awareness [23, 43]. This consolidation stage is a vital part of transitioning from the initial fragile motor representations of the fast learning stage into the more robust, stable forms typical of the slow learning stage [54, 55].

The current study focuses on the initial, fast stage of motor learning. For the consolidation and slow learning stage to be effective, individuals must successfully develop initial motor representations in a format which most effectively primes the associative and sensorimotor parallel circuits for subsequent consolidation processes. As multiple studies have demonstrated enhanced skill stabilization associated with the presence of explicit awareness [23, 43], fast learning resulting in this awareness state may provide a pathway to this primed neural state.

1.2.3 Implicit versus explicit systems in sequential motor learning

Procedural, or sequential motor learning involves the concatenation of discrete movements into a single, fluid motion. Performance gains associated with such sequential learning are often accom-

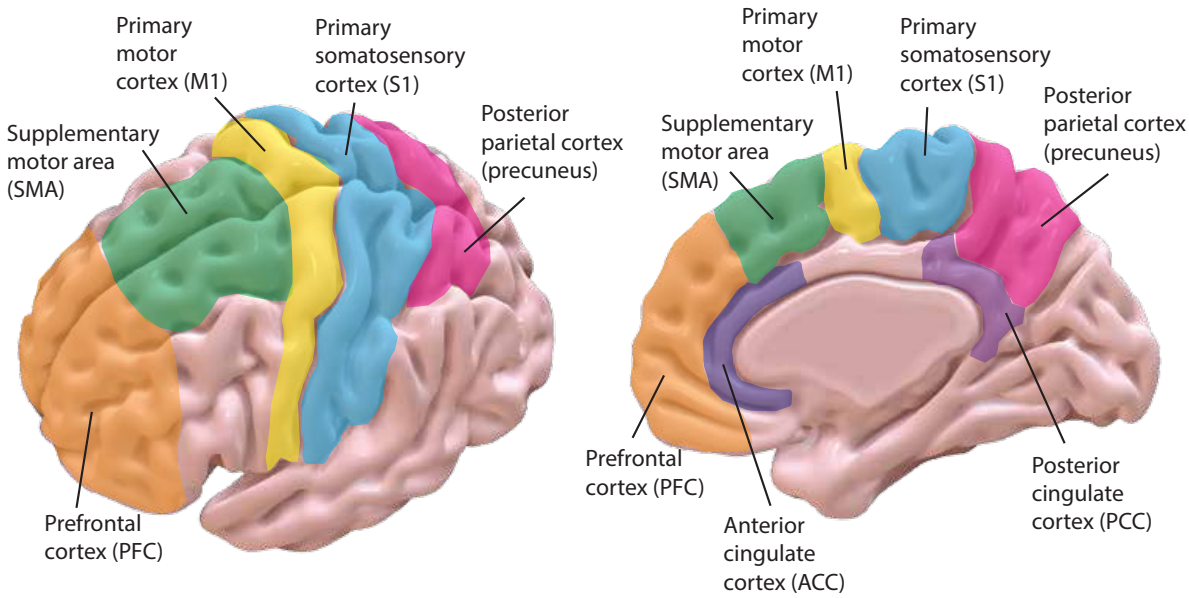
plished without conscious awareness of the individual elements making up the movement. This improvement in movement execution fluidity is suggested to involve the implicit system, while the conscious goal-oriented selection of the sequential movement involves the explicit system [56]. These two systems are proposed to work in parallel during the learning and execution of most sequential tasks.

One common paradigm used to study sequential motor learning is the serial reaction time task (SRTT). The original paradigm involved having a subject match the relative location of a visual stimulus to the location on a response pad [57]. The location of the stimulus varies with each trial, requiring the subject to use a different finger to record the response. Unknown to the subject, however, there is a sequential order to stimuli location. Even without explicit awareness of this embedded sequential pattern, the subjects response time decreases. When subjects are subsequently exposed to a random stimuli presentation, reaction times increase again, providing evidence that the observed improved performance was sequence-related [58]. The improved movement execution is proposed to be reflective of implicit learning of the motor sequence [59, 60, 36]. In addition to utilizing the SRTT paradigm to study the process of implicit learning, it can also be utilized to study explicit learning. Most explicit learning studies utilize an intentional paradigm in which subjects are told that a sequence is present at the start of the experiment. Based on this information, they begin the experiment with a deliberate search, thereby recruiting the explicit system, from the start, to assist in learning the sequential movement. An alternative means in which the explicit system can be recruited occurs when a subject incidentally discovers the presence of a sequence while participating in a typical implicit SRTT paradigm. As multiple studies have demonstrated that the implicit and explicit systems are separate but interacting systems [39, 61, 62, 23], this discovery-based method of explicit learning, which does not recruit the explicit system at the beginning, potentially alters the impact of interactions between implicit and explicit systems during sequential learning.

1.2.4 Neural correlates of the implicit and explicit systems

The implicit and explicit systems have been shown to be separate, but interacting, systems [39, 62, 23]. Figure 1.1 provides a visual representation for many of the areas referenced in this section. Subcortical regions referenced are not included in the image. The implicit system improves motor execution through utilization of strategy adjustments based on error signals involving cortical connections with both striatal and cerebellar circuitry [45, 36, 20]. Initial encoding of movement patterns in implicit learning are suggested to originate in the primary motor cortex [63]. As practice, and learning, continues, activity in M1 decreases, accompanied by increased activity in cortical regions of the supplementary motor area (SMA) and posterior parietal regions (PPC), as well as striatal and cerebellar subcortical structures [64, 65]. This transition is suggested to be reflective of the element-to-element associations, providing the connections between spatial and motor representations necessary for sequence concatenation, and is associated with the typical performance gains seen with implicit learning [66, 63]. Other implicit learning studies have also implicated the medial temporal lobe (MTL), a region known to be involved in memory formation, and prefrontal cortex (PFC) regions, potentially reflective of utilizing working memory resources during the concatenation of sequence components [22, 67, 28, 68, 69, 70]. Finally, sensorimotor striatal and cerebellar subcortical structures are suggested to be involved in consolidative processes and temporal coding for smooth movement execution [40].

The explicit system is often referred to as a strategy-based system, providing a means with which to rapidly strengthen associations between task goals and environmental conditions [20]. Activation of the explicit system during an intentional SRTT paradigm is shown to involve both prefrontal and MTL regions for initial encoding [63], along with associative striatal subcortical regions. As practice continues, and reaction times improve, increased activation of the M1, SMA, PPC, and sensorimotor striatal and cerebellar regions are noted [64, 63]. This progression supports the idea that explicit learning utilizes working memory early on, with movement optimization, connecting spatial and motor representations occurring later [71, 63]. Together, these studies, along with many others, have demonstrated that many of the same neural regions are involved in



(a) Left hemispheric view of relevant cortical areas

(b) Mesial view of relevant cortical areas

Figure 1.1: **Cortical regions of interest for sequential motor learning.** (a) Left hemispheric view of relevant cortical regions. (b) Mesial view of relevant cortical regions.

both implicit and explicit motor learning [64, 69, 72]. The recruitment of similar neural regions suggests that, while the implicit and explicit systems often work in parallel, there is a potential for resource competition when both systems are simultaneously recruited for task optimization.

1.2.5 Limitations of explicit awareness paradigms

Studies examining the development or presence of explicit awareness through the use of intentional, explicit instruction during a motor task have provided insight into the impacts of such simultaneous involvements of the implicit and explicit systems [28, 73, 29, 74, 74, 75, 30, 76, 77, 78]. The changes associated with such intentional explicit awareness are valuable to study, as they are often used in rehabilitative settings, such as when a patient is provided explicit instructions on both the movement goal and the method or strategy with which to execute the desired goal. Results from these intentional explicit strategy studies have demonstrated that the use of intentional, explicit, instructions for development of explicit awareness can result in beneficial

behavioral changes such as decreases in reaction time, changes in movement kinematics, and anticipatory eye movements, attributed to the capability for anticipatory movements once explicit awareness has been developed [28, 73, 29, 74, 75, 30, 76, 77, 78]. However, additional studies have shown that the use of such explicit instruction can have a detrimental aspect, resulting in a motor plan whose performance and execution is negatively impacted by perturbations or secondary cognitive demands in sequential and adaptation studies [23, 24, 25, 26, 27]. In these explicit instruction studies, subjects are provided with both an explicit goal of reaching and responding to target locations as quickly as possible and an explicit strategy for how to achieve this goal, for example by informing subjects that a sequence is present. The development of such intentional explicit awareness has also been shown to reduce generalization, or transfer, to similar but novel tasks [44, 79, 56]. The reduced generalization is suggested to be due to the development of an effector-specific motor plan controlled by the explicit system, which is interfered with upon transfer [80, 20, 18]. The subject understands the associative rules of the current task, but is unable to apply the rules to novel situations [81].

1.2.6 Effects of incidental explicit awareness

While most studies examining the impact of explicit awareness have utilized an intentional paradigm to induce awareness, few studies have examined the impact of incidental development of explicit awareness. Experimental results from such incidental studies have demonstrated positive effects of explicit awareness such as enhanced perceptual sensitivity, enhanced motivational vigor, and improved generalization [28, 73, 82, 31, 83]. These results suggest that incidental development of explicit awareness may provide a mechanism in which patients would have the beneficial behavioral aspects of explicit awareness, while reducing the detrimental effects such as reduced generalization. In a rehabilitative setting, this incidental awareness could occur when a patient is explicitly told to focus on a goal without being provided with explicit instructions on how to achieve the goal. After enough practice, the patient may develop an explicit awareness of the movement patterns utilized for successful accomplishment of the goal.

Recent studies have provided evidence which suggested that, due to initial implicit learning, subjects develop stronger corticostriatal connection during practice, resulting in improved movement execution [84, 85, 86]. The proposed enhanced cortiostriatal connections may be reflective of consolidation-like strengthening of synaptic connections, which may explain why subjects who develop incidental awareness may demonstrate better transfer without the need for offline processing time. Additionally, the utilization of learning via the implicit system, prior to explicit awareness development, may result in enhanced perceptual sensitivity as a result of improved visuomotor communication [87, 88, 89]. In incidental explicit awareness learning, the subject has both improved movement execution from implicit learning, along with the additional benefit of understanding the underlying rules associated with explicit awareness [81]. While these incidental paradigms have revealed significant beneficial impacts of explicit awareness compared to intentional paradigms, they have only examined changes present after awareness has developed through group averaging. Such group analysis fails to address the variation in individual learning rates, potentially masking important, transient, neural correlates involved during the discovery process.

1.2.7 Individual variability in motor learning

Individual variability in motor skill acquisition has long been recognized, but is often ignored, assuming that group averaged learning curves accurately represents individual changes over time [90, 26, 91, 54]. This long-standing practice has potentially limited our understanding of the neurocognitive processes involved in motor learning. By ignoring variability, studies may have inadvertently muted potentially significant results, or even formed misleading interpretations of results due to the group averaging of results [92]. By considering the individual learning rates relative to relevant behavioral parameters, we introduce an opportunity to more precisely examine neural changes occurring with motor learning. This individual examination approach may provide a tool in which to quantitatively measure changes in efficiency for specific cognitive processes during motor learning. Recent studies examining individual variations in motor learning have noted increased functional activations in sensorimotor regions, increased resting state connectivity,

and increased white matter structural connectivity associated with performance gains [93, 94, 95]. While these studies provide correlative measures associated with motor skill performance after learning, they have not examined the changes during the learning process itself. Having a more complete understanding of how these neural regions interact during the learning process may help to better individualize practice patterns for more efficient motor skill acquisition.

In order to individualize the examination of neurobehavioral changes during the learning process, it is necessary to know when an individual has learned. In sequential motor learning studies, the identification of if a subject has developed awareness typically involving recollection methods at the end of the experiment. Identifying when a subject has developed incidental awareness, however, is much more challenging, requiring an indirect measure that does not interfere with the discovery process. Having a method with which to identify the individual timing of awareness development would provide an opportunity to more precisely examine the individualized neural progression associated with such learning.

1.2.8 Neural processes facilitating sequential learning and generalization

Benefits of anticipatory action regulation

The development of sequential motor learning to a level of explicit awareness requires an integration of the visual and motor systems described earlier. Multiple studies have demonstrated dynamic functional connectivity changes between visual and motor networks that are associated with improved motor skill acquisition from a group analysis perspective [96, 97, 98]. These connectivity changes reveal a bidirectional communication and modulation between visual and motor systems during a motor learning task. It is intuitive to recognize the value of visual processing in facilitating motor planning, and in providing precise end-point locations and possible obstacles to avoid for online motor control. What is less intuitive, but recently demonstrated, is the beneficial impact that action preparation has on perceptual processing [99, 100]. The identification of environmental regularities provides opportunities for anticipatory action regulation. Anticipatory action regulation may assist movement quality through preparatory mechanisms prior to movement

execution. Such regulation can be either preemptive or proactive. If preemptive, perceptual processing efficiency is enhanced through selective attention, filtering out task-irrelevant stimuli [101, 102, 103]. If proactive, perceptually driven action control mechanisms are strengthened to suppress or overcome incorrect movements [104, 105]. Both preemptive and proactive mechanisms result in enhanced visuomotor integration for motor planning and control.

Neural correlates of pattern recognition for anticipatory action regulation

The benefits of anticipatory action regulation lead one to ask how information necessary for advanced planning is acquired. The presence of environmental regularities provide a natural substrate for the utilization of probabilistic learning by the nervous system to recognize and take advantage of such regularities for anticipatory action regulation. For probabilistic learning to occur, one must have either a prediction of expected behavioral outcomes, or of perceptual information, with which to compare actual outcome results [89, 106, 107, 87]. The anterior cingulate (ACC) region has been implicated in such performance and error monitoring, as well as in studies examining the identification and utilization of predictable stimuli [108, 109]. Studies focused on the ACC have revealed direct connections with striatal, thalamic, and locus coerulus regions, areas involved in motor coordination and control [110]. These connections are proposed to be part of a network in which the ACC plays a pivotal role in facilitating the transition of explorative behaviors to exploitative, anticipatory actions [108]. Additionally, the posterior cingulate (PCC) has been implicated in reinforcement learning, detecting relevant environmental changes resulting in the transition to, or learning of, new behavioral strategies for optimal performance [111, 112, 113].

The ACC and PCC are hypothesized to rely on sensorimotor information to develop predictions and make comparisons to identify perceptual motor patterns. In a visuomotor task, the perceptual systems utilized will include both visual and somatosensory information. Processing of incoming visual information has been demonstrated to involve a hemispheric specialization within the inferior parietal and precuneus (Pcun) regions. The right precuneus (RPcun) is associated with stimulus encoding and the accompanying visuospatial transformations necessary for eye-hand coordinated

movements [114, 115, 116, 117]. The left precuneus (LPcun) has been shown to be involved in praxis planning along with the encoding and development of future intentions involving visuomotor plans [118, 119, 120, 121]. Proprioceptive information regarding the current location of the limb to move is vital for initial motor planning via the LPcun region. The internal model of motor control proposes that this information is utilized to develop an estimate of expected sensory feedback if the limb movement is accurate [122, 123, 124]. This sensory feedback estimate can then be compared with incoming sensorimotor feedback regarding the end location of the limb. Such sensorimotor information about pre- and post-movement states are processed in secondary somatosensory cortical areas [125, 126, 127, 128, 129]. The supplementary motor area (SMA) is a region known to be involved in the generation of voluntary movements and comparison of sensorimotor information. Anatomical studies have shown the SMA has connections with multiple regions including the precuneus network for perceptual processing and visuomotor planning, the primary motor region for motor execution, the somatosensory network for action outcome proprioceptive information, and the anterior cingulate region for error monitoring [130, 131, 132, 133, 134, 135, 136, 137]. This places the SMA at the center of visuomotor integration during motor skill acquisition and is a key location for activation upon the transition from explorative to exploitive behavior, a necessary shift for the incidental learning of a sequence.

Visuomotor integration resulting in the development of awareness, requires an organized interaction between regions involved in detecting regularity through perceptual processing, sensorimotor comparisons, and error monitoring. One of the primary prerequisites for the development of explicit awareness has been suggested to be the presence of an unexpected event [69]. This unexpected events hypothesis proposes that the recognition of an unexpected event activates a strategy shift which instigates a search for the origin of the unexpected event. This suggests that participants develop an expectation about some upcoming event which is then violated, thus prompting a focused perceptual search. Recent studies examining network interactions during insight development have implicated networks which include both the PCC and ACC [111, 112, 113, 138, 110, 139, 105]. The PCC is noted to decrease under attention-demanding tasks compared to resting

state activity, suggesting it plays a role in the brains underlying intrinsic activity and spontaneous thought processes [140]. Alternatively, the ACC is noted to increase under attention-demanding tasks, being utilized during tasks which require monitoring for inhibitory control [141, 142, 143]. From the perspective of awareness development, the PCC may be pivotal for idea, or strategy, generation, while idea/strategy evaluation involves coupling of the PCC to the ACC [144, 145, 146]. Examination of these networks, along with the temporal progression of activity over sensorimotor areas may provide insight into how the various neural regions interact to facilitate sequence discovery.

1.2.9 Factors contributing to successful visuomotor integration in motor skill learning and awareness development

Multiple factors contribute to the visuomotor integration necessary for the successful concatenation of submovements and subsequent development of explicit awareness. These include: (1) the structure and length of the sequence to be learned, (2) the working memory of the participant, (3) the participants incoming motor skill capabilities due to prior experience and the level of effective neuromuscular connectivity. All of these, separately or combined, may present limiting factors in the ability of a person to explicitly identify and successfully execute the sequence.

The SRTT as an experimental paradigm in which to study motor learning has been around for decades, with many studies examining how factors of the paradigm design itself may influence the development of awareness. A study by DeCoster et al. demonstrated that the presence of triads (ex. 232 or 414) resulted in faster sequence learning [147]. Another study showed that the presence of a pause between sequence elements altered the neural structures involved in the sequence learning compared with a no-pause condition, with the pause condition more extensively recruiting frontoparietal visuomotor regions, along with enhanced striatal, thalamic and cerebellar activations [148]. Finally, multiple studies have revealed the impact of sequence length on the probability of explicit awareness development. Studies exploring the effects of explicit awareness have utilized sequence lengths of 6-8 elements [72, 28, 83, 73]. Studies that reported the presence or absence of

incidentally developed explicit awareness as a secondary measure, suggest that sequence lengths of 10-16 elements result in minimal to no explicit awareness development [149, 54, 59, 150, 147].

In addition to the sequence structure being learned, it has been well established that working memory (WM) plays a pivotal role in the ability to successfully learn and execute a motor sequence. Correlational studies have shown strong correlations between the reduced WM capacity in both aging and autistic populations and the degree of reduced motor sequence learning compared to healthy controls [11, 151]. Seidler et al. demonstrated, through multiple studies, the role of spatial WM in motor learning [152, 153, 154, 155]. These studies propose that spatial WM capacity is necessary for sensorimotor comparisons of upcoming actions with developing predictions based on statistical regularities. Additional studies and reviews have provided further elucidation into when WM may play a more significant role in sequence acquisition. There is evidence that WM capacity plays a larger role for explicit learning compared to implicit learning of a sequence [156, 157]. Additionally, a recent study demonstrated that WM correlations are more clearly demonstrated with sequence-specific learning tasks compared to general skill, or practice effect measures [158].

1.2.10 Clinical relevance

Multiple patient populations with disrupted neuromuscular connectivity have demonstrated reduced motor learning capabilities, including amputation, stroke, and multiple sclerosis [12, 159, 160, 161, 162, 6, 163, 9, 8, 164]. For upper-limb amputees, this reduced motor skill acquisition may be a contributing factor in the high rejection rate (30-50%), or non-functional use of prostheses, in spite of the huge technological advances that have been made with prosthetic devices [165, 166, 167, 159]. Upper-limb amputees are suggested to use poor internal models of the combined residual limb and prosthesis when experiencing perturbations, resulting in large movement errors due to poor sensorimotor sensitivity [166]. Additionally, the proprioceptive errors made when initially learning to use a prosthetic device may divert attentional resources, reducing the ability to learn sequential movements with the device, therefore requiring more practice to acquire the

desired level of motor skill [12]. The frustration that follows may result in the learned non-use phenomenon observed in many patient populations [168]. Identifying effective training methods which may result in enhanced visuomotor sensitivity while enhancing motivation for additional practice, may help to improve the motor skills amputees experience with a prosthetic arm. As the disruption in neuromuscular connectivity and prior motor skill experience is distinctly different for individuals, how much and what type of motor learning will be effective is likely to be different as well. Having a tool in which to compare individual motor learning progression for these patient populations may introduce insights which can assist in delivering interventions most appropriate for each individual.

1.3 Overall goal

Understanding the neural processes occurring in healthy individuals during motor learning may provide insight into not only the mechanisms of learning, but also identify factors that can impede learning. Recent results regarding the impact of explicit awareness developed incidentally during sequential motor tasks such as the SRTT suggest that there may be potential therapeutic benefits of incidentally learned behaviors [82, 31]. However, these studies are limited to a group analysis methodology due to the lack of a reliable, indirect measure for identifying when explicit awareness development occurs. Having an indicator that considers learning rate variability provides an opportunity to examine the neural changes from the individual level, potentially revealing previously unknown, transient neural interactions vital to awareness development. The identification of such an individualized indicator of awareness will also provide the tools to further explore the impact of incidentally developed awareness on attributes vital to successful motor skill acquisition such as generalization and movement quality. Taken together, these results will provide insight into the underlying processes involved in procedural motor skill learning. These insights can then be used to more effectively individualize practice patterns. Aims are summarized below.

1.3.1 Aim 1: Identification of an individualized, behavioral indicator for the presence of incidentally developed explicit awareness.

Previous studies examining behavior of explicitly aware subjects have shown a significant reaction time decrease associated with the presence of awareness [72]. Due to variability in prior experience in visuomotor tasks, how quickly a person adapts to the initial constraints of the task may provide a baseline marker with which to compare future behavioral performance. Therefore, it is hypothesized that a behavioral threshold relative to each individuals baseline performance will be most predictive of the timing of awareness development. Once validated, the indicator was used to identify the neural changes associated with perceptual processing relative to the individual timing of explicit awareness development. Based on previous studies exploring the visuomotor integration associated with motor learning, it was predicted that a facilitative network consisting of PCC, left and right PCun, SMA, and ACC will be correlated with behavior predictive of explicit awareness development.

1.3.2 Aim 2: Evaluate the effect of incidentally developed explicit awareness on the transfer to a novel, more difficult task.

Multiple studies have demonstrated the detrimental effects of too much practice or explicit awareness on generalization, but have done so through the use of group analysis or an intentional paradigm [169, 170, 171, 23, 172, 173]. As previously mentioned, the use of group analysis may mask the impact of transient changes occurring during the encoding of perceptual and motor representations [62, 92]. Additionally, the use of intentional paradigms recruits both the explicit system and the implicit system introducing the potential for conflict during motor execution optimization. With incidental paradigms, individuals have time for the implicit system to optimize movement patterns prior to the recruitment of the explicit system for sequence identification. This suggests that the implicit system would be provided, through repetitive practice, time for enhancement of corticostriatal connectivity typically seen in implicit learning paradigms [36, 174]. This enhanced connectivity may provide a mechanism for improved visual search performance and sen-

sorimotor comparisons to allow for future sequence identification. Therefore, it was hypothesized that subjects developing explicit awareness incidentally will demonstrate greater transfer on the more difficult pattern compared to those subjects failing to develop full, explicit awareness.

1.3.3 Aim 3: Examine the development of of incidentally developed explicit awareness in motor learning during human augmentation.

A previous study in our lab has shown that sequence learning is hindered when using a prosthetic device [12]. Amputees are proposed to have increased visuospatial demands of using the prosthesis itself, potentially interfering with the motor integration typically involved in motor learning [175, 45, 125]. The present aim focused on extending the results from Specific Aims 1 and 2 to examine movement kinematic changes at a more precise level than possible with the typical response pad SRTT paradigm, examining changes in both healthy, intact individuals and individuals using a prosthetic device. As the previous studies suggest an emphasis of visuospatial interference when using a prosthesis [12], eye-tracking may provide insight into the timing and interaction of oculomotor, kinematic and neural changes associated with the development or interference of explicit awareness. Based on previous research in our lab, it is hypothesized that subjects utilizing a prosthesis will demonstrate impaired motor learning due to the increased need for visual attentional resources in guiding the prosthetic to the target location, altering the neural processes utilized for discovery of the environmental regularity.

CHAPTER 2

HUMAN NEUROPHYSIOLOGICAL MEASURES

2.1 Monitoring Changes Associated with Sequence Learning Utilizing EEG

As previously mentioned, multiple fMRI studies have shown that motor sequence learning involves interactions between perceptual and motor cortical regions, along with subcortical structures. While these studies provide strong spatial resolution with which to identify structural areas involved, they lack the temporal resolution with which to understand the perceptual and motor communication changes that occur during the development of explicit awareness, and are correlative measures related to blood flow changes rather than actual neuronal activity. Electroencephalography (EEG), however, provides a highly temporally resolute measure with which to explore the neuronal activity related to perceptual processing changes occurring during motor sequence learning [176, 177].

2.2 Neurophysiology of electroencephalography (EEG)

Electroencephalography (EEG) is noninvasive, direct measure of electrical activity that arises from neuronal activity in the brain. EEG measurements have the advantage of providing better temporal resolution than other imaging techniques such as fMRI (milliseconds vs second) and recording direct neural electrical activity. For EEG, the fluctuating currents produced by neurons can be picked up by electrodes placed on the scalp of a participant and recorded as voltage fluctuations [177]. Neurons produce two types of electrical activity, action potentials and post-synaptic potentials. Action potentials are voltage spikes that traverse the length of the axon, at which point neurotransmitters are released, binding to receptors on the postsynaptic membrane. As ion channels, open or close in response to the neurotransmitters, a graded potential is produced, known as the post-synaptic potential (PSP). The post-synaptic graded potential changes produced simultaneously by

multiple neurons are summed together and recorded as local field potential recordings. It is widely believed that the voltage fluctuations observed in an EEG trace are recordings of these summed post-synaptic potentials, rather than action potentials.

2.2.1 Post-synaptic potentials

How neurotransmitter release results in the post-synaptic potential depends on the ion channels opened by the neurotransmitter and the location of the dendrites responding to the neurotransmitters. The opening of ion channels at an apical dendrite, due to the release of excitatory neurotransmitters, will result in an influx of positive ions into the cell. This produces a net negative charge extracellularly. The voltage flows through the neuron and the positive charge exits the at the cell body and basal dendrites. This current flow results in a dipole directed toward the cell body as shown in Figure 2.1(a). This direction is inverted for inhibitory neurotransmitters, as they open ion channels which result in net positive flow out of the cell at the apical dendrites. The direction of the dipole produced will be influenced by the relative orientation of the activated neuron [178].

2.2.2 Equivalent current dipoles (ECD)

The dipole produced by a single neuron is too small to be recorded by a surface EEG electrode. But dipoles from multiple neurons, activated simultaneously, can sum together with respect to both magnitude and direction, resulting in an averaged dipole termed the equivalent current dipole (ECD) [179]. For the ECD signal to be large enough to be recorded at the scalp, multiple conditions must be met: (1) thousands to millions of neurons must produce a dipole at the same time; (2) the dipoles must be spatially aligned to not cancel each other out; (3) PSPs must arise from the same part of the neuron (apical vs. basal dendrite); (4) PSPs must arise from the same neurotransmitter type (excitatory vs. inhibitory). The pyramidal cells of cortical layer of the brain provides this type of neuronal orientation, and is therefore where most electrical activity picked up by EEG electrodes originate from [177]. Deeper structures, such as the basal ganglia, have neurons which are not oriented in parallel with each other, resulting in reduction of the ECD magnitude, making

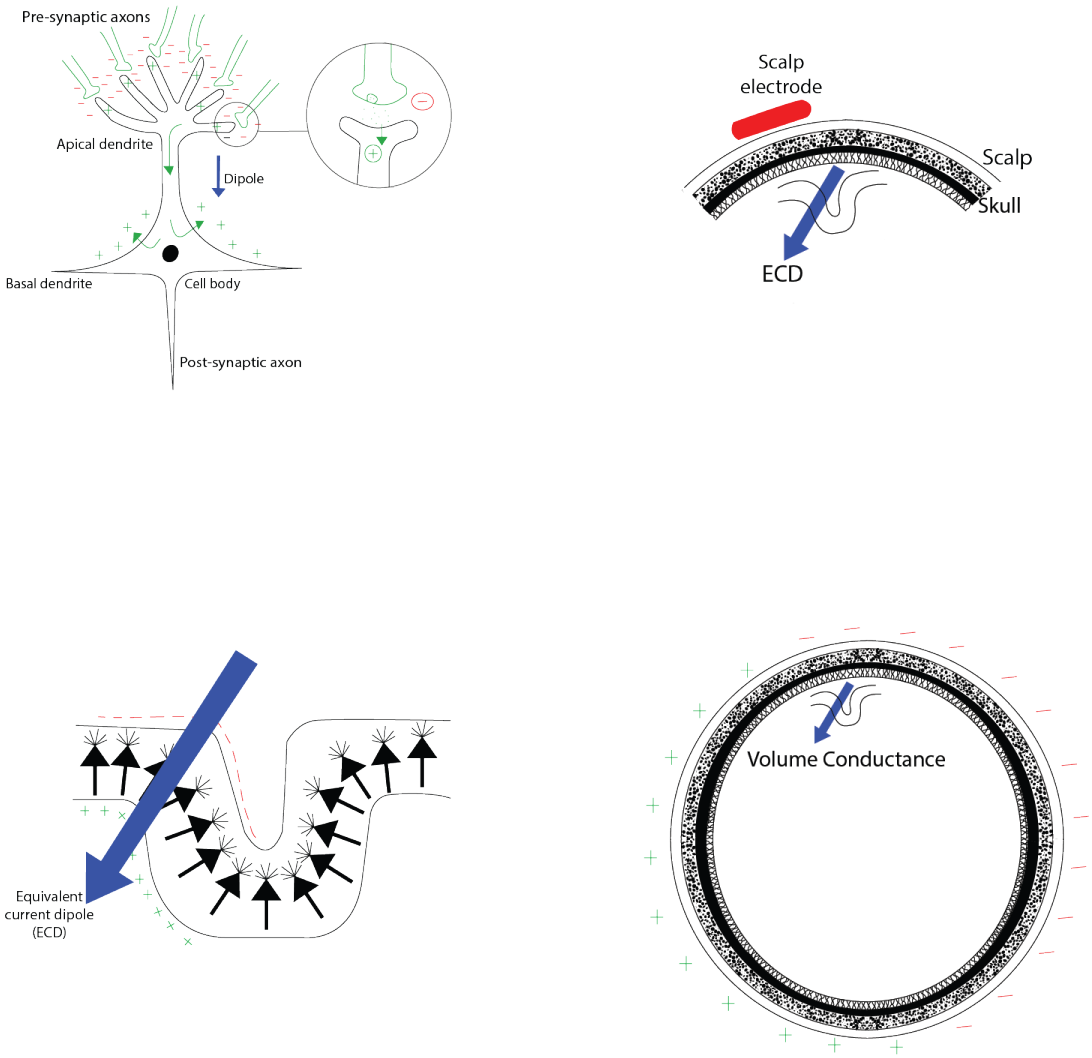


Figure 2.1: **Physiologic basis of EEG measures.** (a) *Post-synaptic potential.* NT release allows positive ion flow inside, resulting in a net negative charge extracellularly. (b) *Dipole production.* Post-synaptic potential of one neuron produces a dipole with magnitude and direction. Voltage flows through the neuron and exits at the cell body region and basal dendrites. Results in a dipole (drawn to +). This direction is inverted for inhibitory neurotransmitters. Direction is also altered for PSPs produced at basal dendrites. (c) *Equivalent current dipole (ECD).* Multiple neurons experiencing PSP graded changes sum together and are recorded as local field potential recordings. Averaged dipole of local field potential provides magnitude and direction of equivalent current dipole (ECD). This measures represents the summed activity of multiple nearby neurons. (d) *Volume conductance.* Volume conductance results in current being transmitted through the brain, picked up by all electrodes with weighting determined by relative location from source.

it much more difficult to pick up with a surface EEG electrode [177, 178, 180].

2.2.3 Electrode placement and naming convention

To increase the reproducibility of EEG studies, a common placement and naming convention for surface electrodes has been established [181]. The original 10-20 system refers to the convention of placing adjacent electrodes at distances of 10% or 20% of the total front to back size of the head. This dissertation utilizes a 58-channel EEG system, which provides for additional electrodes, and, therefore utilizes a 10-10 system, with electrodes being placed 10% from each other. The 10-20 naming conventions typically utilize 1-2 letters and a number to indicate the relative position of the electrode (see Figure 2.2) [182]. The letters F, T, C, P, and O refer to frontal, temporal, central, parietal, and occipital lobes/regions. The numbers refer to the hemisphere in which the electrode is located, with odd numbers indicated the left hemisphere and even numbers referring to the right hemisphere. Smaller numbers are closer to the center of the EEG cap. Electrode designations without a number, but with a Z instead, refer to electrodes located along the midline of the head. In the 10-10 system, the additional use of letters refers to locations relative to the original 10-20 naming convention. For example, a designation of F4A, would be anterior to the frontal electrode F4, while C3P is between C3 and P3. Positioning of the EEG cap is done with reference to the nasion, the depressed area between the eyes above the bridge of the nose, and the inion, the lowest part of the posterior skull which presents a prominent bony projection. The front electrode is placed 10% of this total distance directly above the individuals nasion. This methodology aims to place each electrode over the same anatomical region for all subjects, regardless of head size.

2.3 Event-related potentials / Visually-evoked potentials (VEP)

EEG signals analyzed with reference to a time-locked event, such as the onset of a visual stimulus, are referred to as event-related potentials (ERP) [183, 178]. When the stimulus used is visual, the ERP is typically referred to as a visually-evoked potential (VEP). The EEG signal recorded around the time of stimulus onset will include neuronal activity that is related to the functional processing

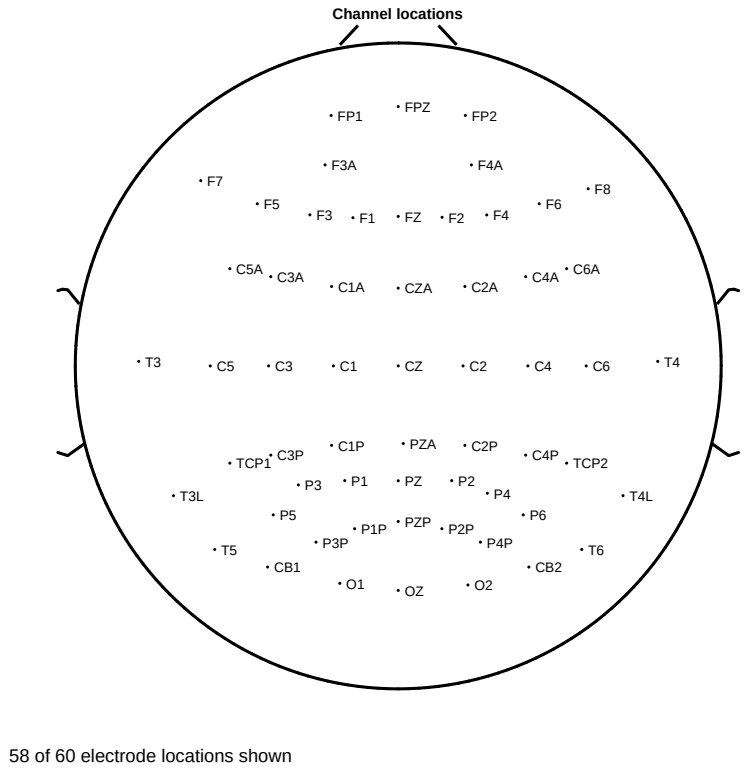


Figure 2.2: **EEG Head Map.** A head map of the electrode locations used in this dissertation. The locations of each electrode follow the international 10-20 system. The nose is at the top of the image and the two extensions laterally indicate the relative location of the ears. Naming convention utilized as: FP=Frontal Polar, F=Frontal, CA=Central Anterior, C=Central, CP=Central Posterior, P=Parietal, T=Temporal, TL=Temporal Lateral, O=Occipital, CB=surface over Cerebellum. Numbers represent the hemisphere each electrode is located on, with even and odd numbers indicating right and left hemisphere electrodes, respectively. Electrodes identified with a Z indicate midline electrodes.

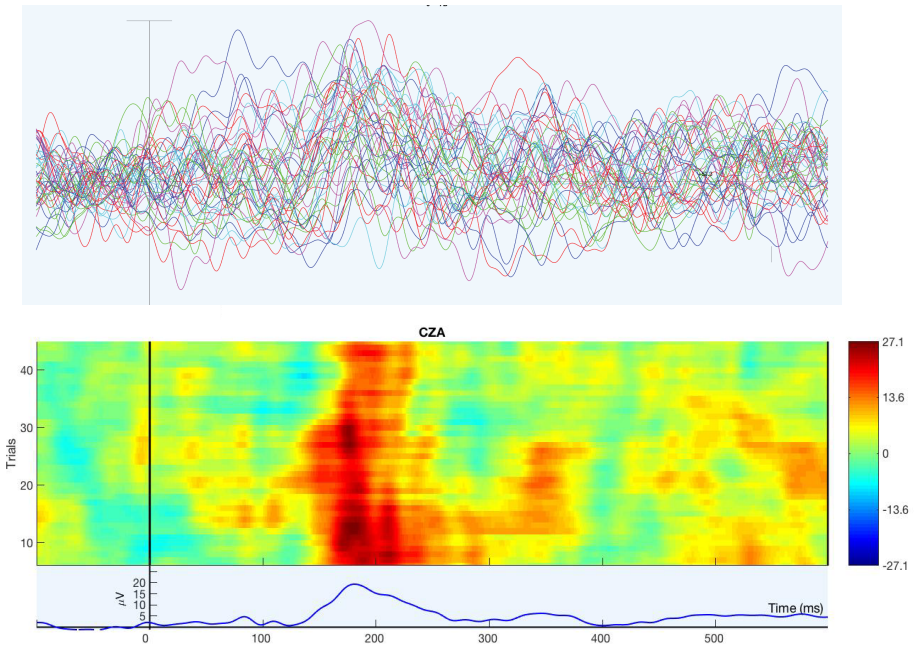


Figure 2.3: **Visually-evoked potentials.** (a) Individual VEP traces for multiple trials plotted on one image. (b) Shows the value of averaging VEP traces to subtract out noise that is not consistent between trials. VEP signal that remains represents the neural activity which was consistently present between trials.

of stimulus information, as well as background signals unrelated to the stimulus, resulting in a signal with a low signal-to-noise ratio. To reduce the impact of the background neuronal activity, multiple VEP trials are averaged together. Neuronal activity associated with the stimulus will be activated in a similar spatial and temporal manner for all trials preserving the signal with averaging, while background activity will tend to be cancelled out or reduced with averaging, producing a signal which more clearly represents the time course of neuronal activity related to the stimulus. Figure 2.3 shows the benefit of averaging multiple VEP trials to produce an average VEP waveform. A typical VEP waveform will show both positive and negative deflections, relative to a baseline period prior to stimulus onset. These peaks and valleys are referred to as VEP components classified by the timing and direction of the voltage deflection from baseline.

There are many contributing factors resulting in a positive or negative deflection including: (1) whether the PSP produced the result of an excitatory or inhibitory neurotransmitter; (2) whether the PSP occur at the apical or basal dendrite; (3) the relative location of the generating dipole to

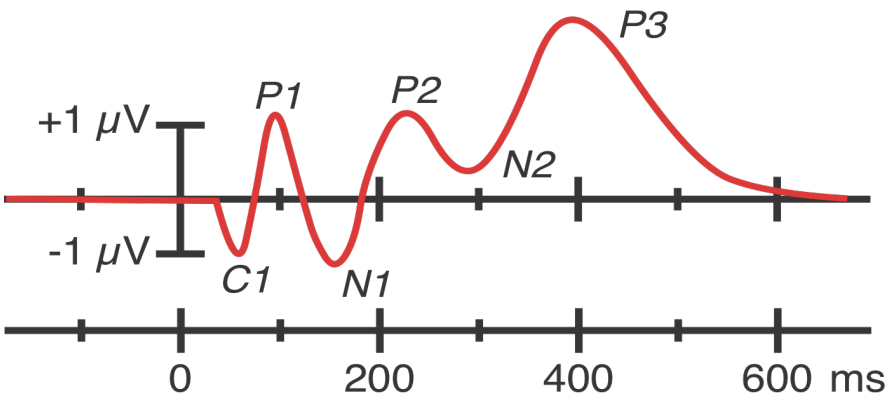


Figure 2.4: **VEP Components.** A pictorial representation of major VEP components, demonstrating both the significance of timing and polarity for naming convention. VEP components with a positive deflection are denoted with a P, while negative deflecting VEP components are designated with an N. The number represents the accepted order of appearance for each VEP component relative to other components with similar deflections. ©S.J.Luck

the recording electrode; (4) the location of the reference electrode [178]. Typically, all of this information is not known, therefore, polarity is not informative for anything other than telling us which VEP component is represented.

Figure 2.4 shows a simplified pictorial representation of a typical VEP waveform. Changes in both the timing and amplitude of VEP components, over specific brain regions, have been associated with unique perceptual and cognitive processes, providing insight into changes in neural communication that occurs with a temporal resolution of milliseconds [184, 178]. The first component typically observed, C1, will demonstrate either a positive or negative deflection, determined by which visual field, upper or lower, is activated by the stimulus, generated in area V1, the primary visual cortex [178, 183]. The next component, P1, is a positive deflection, generated by the extrastriate cortex, peaking around 60-90 ms after stimulus onset. The P1 is thought to reflect the initial stages of perceptual processing.

The P1 component is followed by a negative deflection, N1, peaking between 100-200 ms post stimulus, with peak latency variations based on experimental conditions and electrode location. The N1 demonstrates subcomponents, with the early N1 showing peak latency around 100-150 ms over frontal electrodes, and the late N1 demonstrating peak latency around 140-200 ms over

parietal electrode locations. It has been proposed that the early N1 subcomponent reflects attentional gating mechanisms localized to frontal regions including the ACC [138, 185]. Alternatively, the late N1 subcomponent has been shown to reflect visual perceptual processing neuronal activity associated with attributes such as spatial attention, evidence accumulation and visual search performance [186, 187, 188, 189, 190, 191, 192].

Components following the N1 are most likely associated with higher level, cognitive processing. The P2, a positive deflection following the N1, is typically localized over frontocentral electrodes and peaks around 150-250 ms post stimulus. Recent studies attribute the P2 component to cognitive processes such as target identification, comparison of sensorimotor information, and conscious awareness [193, 194, 185, 195, 196]. The P2 is followed by a negative-going deflection, N2, peaking around 150-200 ms, that is typically seen during oddball experiments leading researchers to attribute its generation to error, or conflict monitoring. The N2 is best isolated when neural activity is time-locked to a subjects response rather than stimulus onset [178, 197]. The N2 is typically followed by a positive deflection, P3, which peaks between 250-450 ms, and has been shown to be prominent over both frontal and parietal electrode locations [178, 183]. The P3 component is also associated with higher level cognitive functions including categorization of visual stimuli, working memory encoding and maintenance, context updating and recognition of behaviorally relevant information [194, 198, 199, 200, 201].

While VEP components are clearly visible after baseline normalization and trial averaging, the neurophysiological mechanisms underlying these components is still under debate. Currently, the two most widely accepted theories are the additive model and the phase reset model [202, 178, 177]. The additive model proposes that the VEP reflects neuronal signals elicited by the stimulus which are simply added onto to the background oscillatory activity. The phase reset model proposes that the VEP signal results from a sudden resetting and alignment of phases of ongoing oscillations increasing the magnitude of the signal and seen as a peak or valley in the VEP trace. While the underlying mechanism is still unclear, the repeated correlative appearance of the components in response to specific perceptual and cognitive processing, make the VEP waveform

a valuable tool for examining neuronal activity with a very precise temporal resolution.

2.3.1 Amplitude and latency calculation considerations

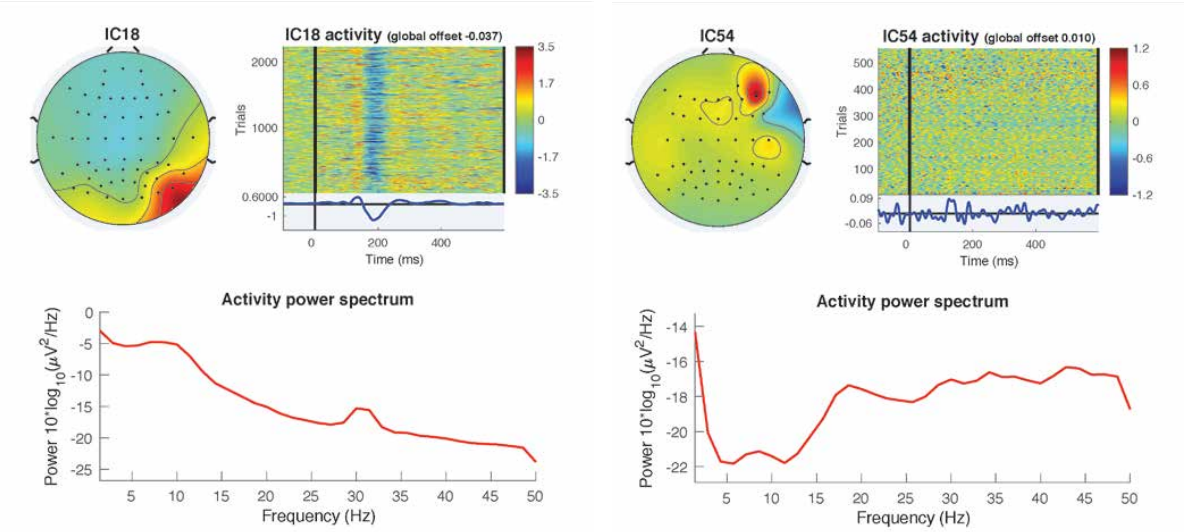
Studies examining the VEP components are typically interested in changed to either the amplitude or latency when exposed to various experimental manipulations. Changes in component amplitudes are typically interpreted as altered levels of neuronal activity or recruitment. Changes in latency, especially for cognitively related components, are often assumed to reflect the level of cognitive difficulty. [178]

Historically, many researchers have relied on the peak voltage of an VEP waveform to determine both the amplitude and latency measures. However, this may not be the most accurate reflection of the neuronal activity represented by the averaged VEP component being analyzed [183, 178, 177]. While each trial will have very similar onset latencies for each component, they will not likely be identical between trials. Averaging will then result in a waveform which may not necessarily demonstrate a distinctive peak. One method recently proposed by Luck et al. to address this issue, is the utilization of a method known as the signed area amplitude calculation [178]. The signed area amplitude method calculates the area under the curve for a given time window, specifically focused specifically on either the positive or negative values of the waveform, depending on the component of interest. As the signed area amplitude is a calculation of the area under the curve for a voltage over time VEP graph, the units of measure for this method is uV*ms. This method presents a major advantage over the typical peak amplitude approach by allowing analysis of a much wider time window without concern for overlapping components, addressing the concern of timing variability that may occur between trials. It does, however, come at a potential cost of being biased toward larger than true values, being more heavily impacted by noisy waveforms. All individual VEP component amplitude analysis in this thesis will, therefore, utilize the signed area amplitude method to assess component amplitude changes during the learning process.

2.4 Artifact rejection Independent Component Analysis

While EEG is known for its high temporal resolution, it is limited in its spatial resolution compared to imaging methods such as fMRI. The spatial resolution of EEG is determined by the number of electrodes, with larger number of electrodes providing greater spatial resolution. The current study utilized a 58-channel EEG cap to provide a reasonable level of spatial resolution. One of the challenges in providing spatial resolution and accuracy is the issue of volume conduction. The brain provides a very conductive medium, resulting in the conduction of current produced by neuronal activity throughout the head [177, 178, 203]. The result of this volume conductance is that the voltage field produced by a dipole generator will not appear at just the electrode directly above, but rather, by electrodes all over the head. Due to the directional nature of the dipole, some electrodes will pick up the positive voltage field, while others will pick up the negative voltage field. Additionally, the position and orientation of the dipole, relative to each recording electrode, along with the conductivity of various tissues in the head, will impact how much of the voltage a particular electrode will receive. Each recorded electrode signal, therefore, represents a weighted sum of voltage signals from all dipole source generators.

The fact that the recording from a single electrode is a complex mixture of signals, or components, from multiple sources introduces a challenge for EEG analysis. Modern analytic techniques attempt to address this issue by assuming the neural signal can be separated into components, each which contributes to the waveform with a certain weight based on factors such as distance from the electrode and conductivity parameters. Statistical methodological approaches such as independent component analysis (ICA) can be used to estimate the most likely scalp distribution of components which best explain the variance in the data of each electrode [203]. This method will produce the same number of components as electrode signals provided. Therefore, a 58-channel EEG signal will produce 58 ICA components. Each component has its own characteristic scalp map topography and frequency distribution. A scalp map topography provides a visualization of the relative voltage magnitudes at each electrode. The frequency distribution can be used to sepa-



(a) ICA component of brain activity

(b) ICA component of signal noise

Figure 2.5: **ICA Components for artifact rejection.** Two examples of ICA components identified for an individual's EEG recording. (a) ICA component which demonstrates scalp topography, VEP and frequency distributions reflective of a neural signal associated with stimulus onset. (b) ICA component demonstrative of signal noise. Note the unusual scalp topography toward the periphery, lack of VEP signal linked to stimulus onset, and the prevalence of high frequency signal.

rate signals that are more likely to represent brain activity (Figure 2.5(a)), compared to signal noise (Figure 2.5(b)). These unique attributes can be used to identify movement artifact signals that may be contaminating the EEG signal, such as eye blinks and head movement. Due to the weighting calculation obtained from the ICA analysis, these artifacts can be subtracted out from the signal of each electrode appropriately, leaving a much cleaner EEG signal.

2.5 Source localization dipole localization methods

While the use of ICA provides a method in which to separate the components producing a particular voltage time-series signal, it does not provide any information regarding the actual source of the signal. If one knows the source generating a dipole of a specific magnitude and direction, the signal detected by a particular electrode can be calculated if the relative locations of the dipole and electrode are known and normative conductive values from tissues such as brain, scalp and skull are assumed [180]. This is known as the forward problem, knowing the source to predict

the signal. Looking at the signal to identify the source, known as the inverse problem, is much more difficult. This is due to the fact that there is not a single solution to the problem. Multiple combinations of dipole generators, when summed together may produce the same electrode, or component signal. Therefore, it is impossible to know with certainty which combination actually represents the source generators for a particular recorded signal [177, 178, 183].

The collection of multiple trials with similar neural activity can provide a method in which to generated source localization with more confidence. Many modern dipole localization algorithms utilize the multiple signals provided to find the dipole source solution which explains the most variance between trials [180]. A common mathematical approach utilizes the forward problem methodology, considering head shape, tissue conductivity, and manipulating potential source parameters, such as dipole location, orientation and strength, to produce expected, or model data. This model data can then be compared against the measured data. Iterations are completed until an optimal solution, which minimizes errors between model and measured data, can be obtained. The resulting solution produces a source estimation for the scalp topography of each independent component.

CHAPTER 3

SPECIFIC AIM 1

3.1 Introduction

The purpose of Aim 1 was to identify an individualized, behavioral indicator for the presence of incidentally developed explicit awareness. As previously described in Chapter 1, the presence of incidentally driven explicit awareness in sequential motor tasks, such as the Serial Reaction Time task (SRT task), is associated with beneficial performance changes such as enhanced movement vigor, as well as improved perceptual sensitivity and generalization [28, 73, 82, 31, 83]. While these studies have provided insight into the effects of incidental explicit awareness, they have not examined the changes associated during the learning process itself. It is known that variations in skill learning occur, often attributed to a subject's initial state, this variation could result in time course differences in awareness development, potentially hiding valuable information when reporting group averages [75, 92]. The current Aim addressed this concern through examination of learning-dependent changes normalized to each individual's point of discovery.

Such an approach necessitates the use of an indicator which detects, not only if awareness is present, but also when it occurred. This, however, is not a trivial task, as the indicator must be one which can detect awareness onset while avoiding interference with the incidental nature of discovery. Therefore, the indicator must be determined indirectly through behavioral measures such as changes in response latencies. Prior studies approached this challenge utilizing a number reduction task (NRT) paradigm. The NRT paradigm typically involves a repeating pattern with random stimuli for all presentations except the last one. Once the pattern is discovered, the subject is able to predict the answer, or location, of the last stimuli in the sequence. Subjects with explicit awareness of the NRT pattern demonstrated a dramatic reduction in reaction time [204, 28]. This RT-decrease was found to be predictive of when a subject developed insight into the

pattern, allowing for anticipatory responses to the predictive stimuli, and was used as a marker for examining events prior to and at the moment of insight. While providing valuable information into the development of insight, the authors noted that this method may not be transferable to the SRTT due to explicit awareness of the full sequence coming in chunks, requiring multiple iterations of chunking before the entire sequence is completely explicitly aware. One investigation has utilized the RT-drop approach in a variation of the SRTT to predict when a subject initially became aware of a chunk of the sequence [73]. However, for full explicit awareness of the sequence, the subject must put together multiple chunks. To explore the impact of incidental learning on factors such as generalization and consolidation, it is necessary to identify a predictor of when the entire sequence is explicitly known.

As noted, the presence of awareness promotes a behavioral strategy shift allowing for anticipatory movements, resulting in the observed faster overall response latencies [17]. In addition to these behavioral changes, multiple studies have demonstrated that there are specific neural changes associated with explicit awareness. This strategy change may be reflected neurally as a shift from an externally-guided network, relying on visual stimuli for motor execution, to a more internally-guided network due to knowledge of upcoming response locations. Additionally, as explicit awareness development has been shown to result in improved perceptual sensitivity [82], it is expected that awareness will also involve alterations in the perceptual processing of visual stimuli. Therefore, a reliable behavioral indicator of awareness would be one which demonstrates a significant temporal correlation with these neural changes.

To this end, Aim 1 utilized a two-experiment approach for the identification, and validation of an individualized indicator for the presence of incidentally developed explicit awareness. The first study utilized behavioral data to formulate a predictive model for the presence and timing of explicit awareness. Results from the first study were then validated with a second group of subjects, both behaviorally and neurally. Neural measures employed event-related EEG analyses to examine perceptual activation changes occurring during sequential learning to provide neural assessment of the proposed behavioral predictor.

3.2 Hypotheses

Experiment 1: Identification of individualized indicator. Previous studies have demonstrated the impact of sequence length on the presence of incidental awareness and have shown that sequence lengths of 6-8 result in awareness, whereas sequence lengths greater than 10 result in minimal, to no, explicit awareness [83, 73, 149, 54, 59, 150, 147]. Therefore, it was hypothesized that exposing subjects to varying sequence lengths will ensure behavioral data for subjects both with, and without, awareness with which to develop a predictive model. As awareness of the sequence will provide subjects the opportunity for anticipatory responses [17, 31], it was predicted that a consistently dramatic decrease will be predictive of full explicit awareness. Due to anticipated variability in a subject's baseline sequential learning experience, it was additionally hypothesized that examining such decreases in reaction time relative to baseline performance will provide the most valid predictor for the timing of when awareness is present [75].

Experiment 2: Neurobehavioral validation of individualized indicator. Previous studies examining neural regions involved in implicit sequential learning have demonstrated a frontoparietal network including sensorimotor, parietal and premotor cortices [36], with prefrontal and supplementary motor areas being additionally recruited for explicit learning and awareness [59]. Deeper structures such as basal ganglia, thalamus and cerebellum have also been shown to be modulated during motor learning. As neural activity from deeper sources are not typically available with EEG, changes occurring in these deeper structures were not examined in this dissertation. Upon development of explicit awareness, it was hypothesized that neural changes in the frontoparietal network, seen for explicitly aware subjects, will correlate with behavioral changes identified by the indicator that are not present for subjects failing to develop awareness.

3.3 Experiment 1 Materials and Methods: Model Development of Predictive Indicator

3.3.1 Subjects:

Thirty right-handed (age 18-31 years; 13 females), neurologically healthy adults were recruited to participate in the study. All subjects provided written, informed consent, and the Georgia Institute of Technology Institutional Review Board approved all methods. Subjects completed an Edinburgh Handedness Inventory [205] to assess the level of handedness along with a short questionnaire regarding any previous musical training they had received. Only subjects with a handedness score greater than 0.6 (indicating right hand dominance), and less than 3 years formal musical training, were included from the study.

3.3.2 Experimental Paradigm

Subjects were randomly placed in one of three groups, each experiencing a different number of elements of a sequence: (1) 7-element sequence (7-KEY), (2) 10-element sequence (10-KEY), and (3) 13-element sequence (13-KEY). Previous studies examining incidental awareness have utilized sequences of 6-8 elements in length, while studies examining motor learning without awareness utilized sequences greater than 10 elements [28, 83, 31, 54]. Therefore, sequence lengths of 7-, 10-, and 13-elements were utilized to ensure some subjects will develop awareness, while other subjects remain unaware of the sequence during the study. Due to the focus on the development of incidental explicit awareness, a within-subject design between sequence lengths was not possible due to the potential confound of expecting a repetitive pattern after the first sequence exposure. The sequences were generated in a pseudorandom fashion by a MATLAB code (Natick, MA) to ensure that no consecutive sequence elements were repeated and 3 consecutive fingers would not be used in the sequence. A modified serial reaction time (SRT) task was used, with no random sequence at the end of the paradigm to allow for sequence recall assessment. Visual stimuli consisted of four white rectangles equally spaced in a horizontal manner on the screen. To start the experiment, one of the four rectangles would change from white to red. Subjects were asked to match the

relative location of the red rectangle to the location on the 4-button response pad as quickly and accurately as possible. (Note: Subject instructions were delivered by a single experimenter for all three Aims. This method ensured a consistent delivery of instruction with reference to wording and inflection.) Using their right hand to respond, this corresponded to subjects using the index finger for the far most left rectangle and the far most right rectangle matching to the little finger. Stimulus presentation and timing was managed utilizing STIM2 software from Compumedics Neuroscan^{TM} (Charlotte, NC). Subjects had 750 msec in which to record a response, at which time the next visual stimuli would immediately appear providing an inter-stimulus interval (ISI) of 0 msec. Subjects in the 7- and 13-element conditions were exposed to 182 stimuli per block, with the 10-element condition receiving 180 stimuli per block. This provided each condition a complete number of sequences within each block. Subjects experienced a total of 5 blocks, with a 30 second rest between blocks. 4 seconds before the end of the rest period, subjects received a 3-sec "Get Ready" visual cue and a 1-sec fixation cross, followed by the next block of visual stimuli. Online data collection included latency and accuracy of each response. Upon completion of the paradigm, subjects were asked if they noticed a pattern within the presentation. If they answered yes, subjects were asked to replicate the pattern noticed. Replication responses were recorded to assess for accuracy. Subjects able to repeat the sequence with 100% accuracy were identified as explicitly aware, EXP. As some subjects may learn the sequence at a different starting point than what was presented, accuracy was not limited to the presented starting point. For example, a subject experiencing the 7-element sequence 3-4-2-1-3-2-4, would be classified as EXP if their recall was 3-4-2-1-3-2-4 or if it was 2-1-3-2-4-3-4. Subjects unable to repeat the sequence with 100% accuracy were categorized as not explicitly aware, NOEXP for the purpose of model development. Offline analysis examined latency and accuracy changes for all subjects throughout the experiment. Individual subject data was averaged for each sequence repetition, consisting of 7-, 10-, or 13-key presses.

3.3.3 Statistical Analyses of Behavioral Data

Individual subject data was analyzed utilizing a custom MATLAB (Natick, MA) program. Subject data was first separated into sequence repetitions. To ensure an equal number of sequences for analysis between conditions, analysis was only conducted for the first 70 sequence repetitions, which represented the maximal number of repetitions experienced by the 13-KEY condition. Removal of anticipatory or late key presses (an average of 15% of responses) allowed for analysis of only recorded responses for both accuracy and latency changes. Individual average accuracy and latency of recorded responses for each trial was calculated and utilized for group comparisons. Individual subject baseline performance was calculated as the mean of the first 45 key presses, with the standard deviation being used as a variable in examining predictive thresholds. A repeated-measures analysis of variance (ANOVA) was conducted, utilizing R stats [206], for both accuracy and latency, with SEQ (70 levels) as within-subject, repeated measures variables and CONDITION (3 levels) and AWARENESS (2 levels) as between-subject variables, with significance levels reported at $p < 0.05$, and Holm correction for multiple comparisons. When sphericity was violated, Greenhouse-Geisser corrected p-values are reported.

3.3.4 Development of a Model for Individualized Threshold Predictive of Explicit Awareness

Individual performance at baseline, defined as the first trial of 45 keys, was utilized to calculate the individualized threshold. Behaviorally, a subject's initial state has been attributed to variations in skill learning suggesting a need to individualize the indicator [75]. Therefore, neurobehavioral changes relative to individual baseline performance was hypothesized to provide a stronger basis for evaluating correlates to incidental explicit awareness. Behaviorally, it was expected that subjects demonstrating latencies dramatically different than baseline behavior would be indicative of the presence of awareness of the sequence. For this reason, the behavioral threshold was calculated based on a subject's baseline mean and standard deviation, allowing for a z-score threshold analysis. The formula utilized to test the reliability of an individualized threshold in classifying subjects as having explicit awareness is shown in Eq 3.1

$$\text{Threshold} = Mean_{\text{baseline}} - (\textit{z-score}) * (SD_{\text{baseline}}) \tag{3.1}$$

A customized MATLAB program was then utilized to classify subjects as having explicit awareness (EXP) or no explicit awareness (NOEXP) based on their mean performance for each sequence repetition (ie. 7 keys for the 7-element, 10 keys for the 10-element and 13 keys for the 13-element condition) over the course of the experiment compared to the calculated threshold. To improve reliability of using the subject mean value for each trial, the upper confidence interval was determined as the sequence behavior in which to compare against the calculated threshold. If a subject's upper confidence interval for a sequence repetition dropped below the calculated threshold at any point during the experiment, the classifier would identify the subject as EXP. If the upper confidence interval did not drop below the calculated threshold at any point during the experiment, the classifier identified the subject as NOEXP. The classification results were then compared to the actual experimental results for EXP and NOEXP subjects to determine the sensitivity, or true positive rate (TPR), and specificity, or true negative rate (TNR), of the classifier for that z-score value. Calculations for sensitivity and specificity are shown in Eq 3.2 and 3.3 respectively. Z-score values ranging from 0.5 to 2.5, in increments of 0.01, were examined to identify the most reliable z-score in predicting a subject as having explicit awareness. Sensitivity/specificity analysis was then also conducted utilizing an additional constraint of performing for two consecutive sequence repetitions below threshold for each z-score value to examine the predictive efficacy of a consistency requirement:

True positive (TP) = accurate EXP classification

False negative (FN) = inaccurate NOEXP classification

True negative (TN) = accurate NOEXP classification

False positive (FP) = inaccurate EXP classification

$$\text{Sensitivity(TPR)} = \text{TP}/(\text{TP} + \text{FN}) \tag{3.2}$$

$$\text{Specificity(TNR)} = \text{TN}/(\text{TN} + \text{FP}) \tag{3.3}$$

3.4 Experiment 2 Materials and Methods: Neurobehavioral Assessment of the Individualized Threshold Model

3.4.1 Subjects:

An additional twenty-one right-handed (age 18-32 years; 9 females), neurologically healthy adults were recruited to participate in Experiment 2. All subjects provided written, informed consent, and the Georgia Institute of Technology Institutional Review Board approved all methods. As in Experiment 1, subjects completed an Edinburgh Handedness Inventory [205] to assess the level of handedness along with a short questionnaire regarding any previous musical training they had received.

3.4.2 SRTT Behavioral Task

Based on the results of Experiment 1, Experiment 2 focused solely on the 7-element sequence. Subjects experienced the same sequence as those in Experiment 1, but a customized MATLAB program was written utilizing PsychToolBox [207] for presentation to allow for the recording of anticipatory responses and stimulus markings necessary for EEG epoching. A small black box appeared in the upper and lower left corners of the screen to allow for a photodiode impulse to be detected by StimTracker™ (Cedrus Corporation, San Pedro, CA). A pulse was transmitted both at the onset of each stimulus and at the beginning of each block. This adjustment provided the opportunity to record all responses from EXP subjects and had the potential to result in mean se-

quence latencies that were negative relative to the stimulus onset. Behavior analysis focused on latency and accuracy of responses. Based on observations from Experiment 1, a 35-key random sequence was presented in the first block and utilized to calculate each subject's individualized threshold. Based on the sensitivity/specificity examination in Experiment 1, threshold calculations were made based on a z-score of -1.85. Following the baseline block, subjects experienced 26 blocks consisting of (5) repetitions of the 7-element sequence. This provided a total of 130 sequence repetitions to match the number of sequence repetitions presented in Experiment 1. The stimulus presentation time of 750 ms and ISI of 0 ms was also maintained for consistency. Subjects experienced a 10-second rest period between blocks, with a 30-second rest period after blocks 10 and 20. Upon completion of the experiment, subjects were asked if they recognized a pattern during the experiment and then asked to replicate the pattern. As in Experiment 1, subjects able to replicate the sequence with 100% accuracy were identified as EXP, while those unable to replicate the sequence with 100% accuracy were identified as NOEXP.

3.4.3 EEG Recording and Pre-Processing

EEG analysis utilized a 58-channel EEG cap (Electrocap, Eaton, OH) that recorded scalp potential activity (1000 Hz sampling rate, filtered at DC-100 Hz) via the SynAmps II^{TM} data acquisition system (Compumedics Neuroscan, Charlotte, NC). Data were referenced to the ear electrode and impedances kept below 5 k. The raw, continuous EEG data was then imported into MATLAB's EEGLAB [203] for pre-processing and analysis. Data was first high-pass filtered at 0.5 Hz, bad channels were identified, rejected and interpolated. Data was then epoched from 100 ms pre- stimulus onset to 500 ms post-stimulus for all 945 stimuli presentations with baseline correction from 100 to 0 msec, to provide epochs for subsequent visually-evoked potential (VEP) analysis. An independent component analysis (ICA) was then conducted, utilizing EEGLAB's *runica* algorithm, to assist in removal of blink and other stereotypical movement artifact components. Selection of components for removal was based on visual inspection of scalp map localization, unusual spectral frequency patterns and irregular VEP-image activity. An average of 7 of 58 components per partic-

ipant was selected for subtraction. After removal of artifact components, data was segmented into 27 separate datasets, one for each block. A separate dipole fitting analysis was then conducted, utilizing EEGLAB's DIPFIT plugin [180], for every block for each subject. Dipole localization was determined utilizing the Talairach Client application [208]. After pre-processing, all datasets were loaded into a STUDY structure for group analysis.

3.4.4 Behavioral Validation of Threshold Model

Post-experimental analysis converted raw latency values to individualized z-scores both for awareness classification and statistical comparison between subjects. A customized MATLAB program was utilized to calculate the sequence repetition at which a participant had achieved two consecutive repetitions at a performance level below their individualized threshold z-score value of -1.85 and were classified as EXP. Participants failing to drop below threshold were classified as NOEXP. A sensitivity/specificity analysis was again conducted comparing actual vs. predicted classification of awareness. In addition, for EXP subjects, the block at which behavior dropped below threshold was identified as the block of interest for EEG correlation analysis, while the block of lowest z-score performance was utilized as the block of interest for the NOEXP subjects. Subjects who demonstrated full recall without dropping below threshold were identified as false negatives (FN) and excluded from further statistical analysis due to the low number of subjects in this category.

3.4.5 EEG Statistical and Correlation Analysis

Subject datasets were loaded into EEGLAB Study design with identification of the between subject group classification of awareness (EXP vs. NOEXP) and within subject condition of time (Blocks 1-27). Group analyses were conducted by first grouping the data into 9 sections of 3 blocks each. VEP group measures were precomputed utilizing the baseline of -100 to 0 ms. A repeated measures ANOVA was conducted, utilizing permutation statistics with false discovery rate (FDR) correction for multiple comparisons (alpha=0.05), with BLOCK (27 levels) as within-subject, repeated measures variables and AWARENESS (2 levels) as between subjects variables.

AWARENESS categories consisted of EXP (100% recall and correctly classified as EXP by the previously defined threshold) and NOEXP (¡100% recall and correctly classified as NOEXP by the threshold). Specific electrode locations were determined by first examining the VEP scalp map distribution of time periods based on prior work for the VEP components of interest [178]. These post-stimulus time periods included: (1) early N1: 100 to 150 ms, (2) late N1: 150 to 200 ms, (3) P2: 150 to 250 ms, and (4) P3: 250 to 400 ms. Upon examination of the scalp map distributions, specific electrode locations were selected based on the parieto-frontal network noted to be involved in visuospatial motor tasks through multiple studies [209, 210, 193, 211, 197]. These electrode combinations included: (1) early N1 frontal electrodes (F1, FZ, F2), (2) late N1 parietal electrodes (P1, P3, P2, P4), (3) P2 left frontocentral electrodes, due to the right-handed nature of the task (C1A, CZA, C1, CZ), and (4) P3 centroparietal electrodes (C1, CZ, C2, C1P, PZA, C2P). Time ranges utilized in statistical analysis were narrowed post hoc based on the range limits demonstrated by subjects for each component of interest. This resulted in the following time windows: (1) early N1: 100-140 ms, (2) late N1: 140-170 ms, (3) P2: 180-210 ms, and (4) P3: 275-375 ms. Component amplitude values for each block were determined utilizing the signed area amplitude method [178]. The method of signed area amplitude was utilized to address the possible confound of amplitude magnitude differences on subsequent components of the VEP. Corrections for multiple comparisons in the group analysis of VEP components were conducted utilizing EEGLAB's false discovery rate and permutation sampling method. Adjusted p values are reported in results.

As the behavioral indicator provided a prediction of awareness for a specific block, correlations between individual behavioral performance and neural amplitude for each VEP component were conducted with data from all 27 blocks. The behavioral performance variable, for EXP subjects, was the block at which behavior was indicative of awareness by the predictive indicator. As NOEXP subjects had no behavior indicative of awareness, the block at which the lowest z score was observed was utilized as the behavioral performance variable. The neural behavior variable was the block at which the largest amplitude occurred. As the P3 amplitude has been noted to be influenced by pacing attributes [198], it was expected that an initial peak would occur during the first 2 blocks

relative to adaptation of the stimulus-response pacing, Therefore, analysis of changes in peak amplitude was limited to Blocks 3-27. A Spearman correlation analysis was then conducted for peak amplitude relative to behavioral performance for each component. An additional correlation for P300 amplitude vs. response latency over the course of the experiment was conducted to explore the correlation of behavioral latency on the P300 peak amplitude for both EXP and NOEXP groups utilizing a linear mixed model. Fixed effects were behavioral latency and awareness, with an interaction term, and random effects of intercepts for subjects. Correlation analysis was conducted utilizing R statistics packages *rcorr* and *lmer* [212]. Multiple comparisons for correlations were addressed utilizing Holm corrections and adjusted p values reported.

3.5 Results: Experiment 1

3.5.1 Subject Demographics

There was no significant difference in age, handedness, or years of previous musical experience between sequence conditions or awareness classification (see Table 3.1).

Table 3.1: **Subject Demographics.**

Condition	**Age (yrs)**	**Handedness**	**Formal Musical Training (yrs)**
$7 - Element$	22.0 ± 1.01	0.88 ± 0.04	1.5 ± 0.40
$10 - Element$	22.3 ± 1.45	0.88 ± 0.04	1.2 ± 0.42
$13 - Element$	22.0 ± 1.41	0.90 ± 0.03	1.7 ± 0.56
$Experiment2 : EXP$	19.8 ± 1.23	0.92 ± 0.10	1.3 ± 1.33
$Experiment2 : NOEXP$	21.4 ± 3.95	0.97 ± 1.33	1.2 ± 1.33

3.5.2 Effect of Sequence Length: Accuracy

The mean accuracy for recorded responses was $97.93\% \pm 6.61\%$. One subject from the 13KEY condition experienced problems with the response pad for multiple blocks throughout the experiment. Therefore, the 13KEY condition data analysis is restricted to 9 subjects. Two additional

subjects, one from the 7KEY and one from the 10KEY, each had one sequence repetition, repetition 13 and repetition 15, respectively, which did not record multiple responses. The mean latency from the previous repetition was, therefore, used to represent the missing data for these subjects. A repeated measures ANOVA of accuracy for SEQxCONDITION, showed no significant difference for sequence repetition (F(69,1799)=1.048, p=0.373) or between conditions of sequence length (F(2,1799)=0.963, p=0.382) as shown in Table 3.2.

Table 3.2: **Mean baseline latency & accuracy by sequence lengths**

Measure	**7-Element**	**10-Element**	**13-Element**
$BaselineLatency(msec)$	458.69 $\pm$ 51.76	451.80 $\pm$ 34.61	438.69 $\pm$ 28.09
$Accuracy$	98.3% $\pm$ 0.27%	98.08% $\pm$ 0.29%	97.94% $\pm$ 0.54%

3.5.3 Effect of Sequence Length: Latency

The mean latency for accurate recorded responses, at baseline, for all subjects, was 453.41 $\pm$ 29.42 msec, with an average final latency of 346.46 $\pm$ 68.35 msec. The baseline latency performance for all subjects showed no significant difference between conditions. In line with previous SRTT studies [15, 57, 54], all subjects demonstrated a decrease in z-score, relative to baseline latency, over the 70 sequence repetitions experienced. Repeated measures ANOVA for CONDITION, with Greenhouse-Geisser correction, indicated a main effect of sequence repetition for change of z-score (F(69,1799) = 3.908, $p<0.001$), a main effect of sequence length (F(2,1799)=53.368, $p<0.001$), with no interaction effect of sequence length condition for z-score change over the 70 sequences for conditions, (F(138,1799) = 0.547, p=1). Simple main effects analysis showed significant effects ($p<0.05$) appearing between 7-element and 10-element conditions by sequence 69, between 7-element and 13-element by sequence 9, and between 10-element and 13-element conditions by sequence 21.

3.5.4 Effect of Explicit Awareness

Eight subjects were able to correctly replicate the sequence at the end of the experiment and were identified as having explicit awareness, EXP (7 subjects from the 7-KEY condition; 1 subject from the 13-KEY condition). Twenty-one subjects were unable to correctly replicate the entire sequence and were identified as having no explicit awareness, NOEXP. Explicit recall by sequence length summary is shown in Table 3.3.

3.5.5 Effect of Explicit Awareness: Accuracy

Accuracy of recorded responses was 98.10% $\pm$ 0.28% for the EXP subjects and 98.24% $\pm$ 0.33% for the NOEXP subjects. A repeated measures ANOVA of accuracy for SEQxAWARENESS showed no significant difference of sequence repetition ($F(69,1869)=1.045$, $p=0.379$) or between conditions of awareness ($F(1,1869)=0.541$, $p=0.460$).

3.5.6 Effect of Explicit Awareness: Latency

There was no significant difference between EXP and NOEXP conditions for baseline performance ($F(27,1)=3.863$, $p=0.0597$, EXP = 471.12 $\pm$ 34.26 ms, NOEXP = 442.10 $\pm$ 38.76 msec). Fig 3.1 shows the z-score change in latency by awareness, demonstrating the significant difference in response times for subjects expressing EXP recall. Repeated measures ANOVA for AWARENESS, with Greenhouse-Geisser correction, demonstrated a significant main effect of sequence repetition for z score ($F(68,1869)=4.897$, $p<0.001$), a significant main effect of awareness ($F(1,1869)=389.749$, $p<0.001$) and a significant interaction effect ($F(68,1869)=3.245$, $p<0.001$). Simple main effects analysis showed significant effects ($p<0.05$) appearing between EXP and NOEXP subjects by sequence 37, continuing for the remainder of the experiment.

3.5.7 Reliability of the Individualized Threshold in Predicting the Presence of Explicit Awareness

The results of the sensitivity/specificity analysis for the threshold model are in shown in Fig 3.2. Fig 3.2a shows results when utilizing the first occurrence of behavior below the individualized

Table 3.3: **Percent recall by sequence length**

Condition	Number of EXP Subjects	Percentage of Recall for NOEXP Subjects
$7 - Element$	7/10	$42.86\% \pm 11.66\%$
$10 - Element$	0/10	$17.00\% \pm 21.93\%$
$13 - Element$	1/10	$7.69\% \pm 14.50\%$

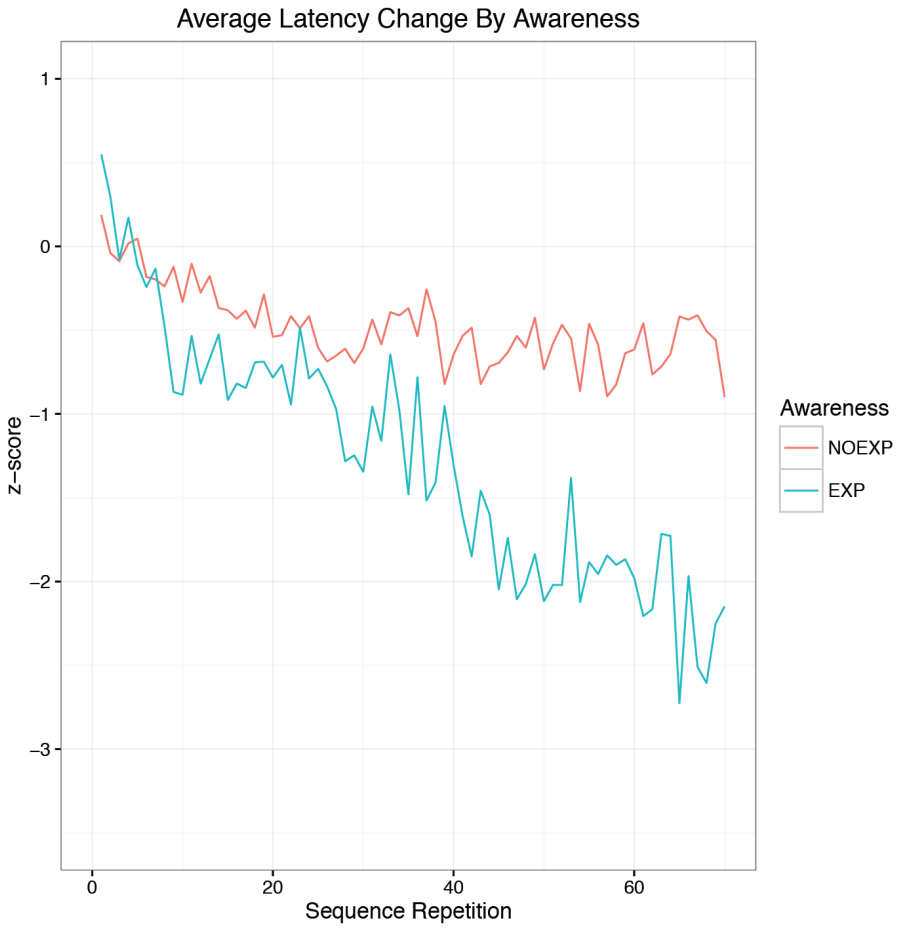


Figure 3.1: **Latency change by awareness.** Latency change over time for subjects with (EXP) or without (NOEXP) sequence awareness.

threshold, while Fig 3.2b shows the requirement of two consecutive sequence occurrences below threshold. There is a distinct improvement in the rigor of accurately classifying a subject as EXP when the threshold model requires two consecutive sequence occurrences below threshold. Utilizing this model, it was noted that a minimum z score of -1.85 was required to provide a 100% sensitivity and specificity in accurately classifying a subject as EXP or NOEXP.

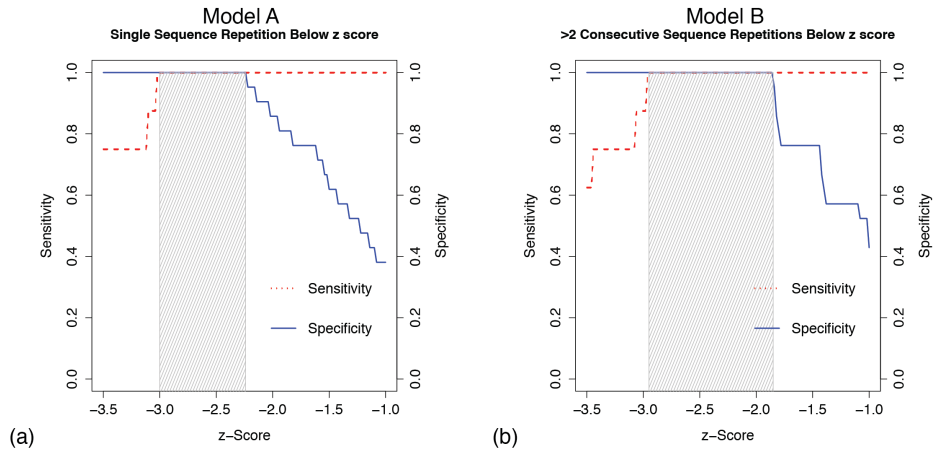


Figure 3.2: **Sensitivity/Specificity plot for threshold model.** (a) Classification of EXP based on the first occurrence of a sequence repetition with 95% confidence level performance below threshold. (b) Classification of EXP based on two consecutive occurrences of a sequence repetition with 95% confidence level performance below threshold.

3.6 Results: Experiment 2

3.6.1 Behavioral Results: Reliability of Threshold Model

Eleven (11) subjects successfully recalled the sequence, with nine of them correctly identified as explicit (EXP) by the threshold model and two subjects incorrectly classified as non-explicit, giving a false negative classification (FN). The remaining ten (10) subjects were unable to accurately recall the sequence and were correctly identified as non-explicit (NOEXP). This results in a sensitivity of 81.8% and specificity of 100% for the individualized threshold model.

3.6.2 Behavioral Results: Accuracy

Accuracy of the baseline block was 95.87% $\pm$ 3.23% for the EXP group and 96.86% $\pm$ 3.42% for the NOEXP group, while the overall accuracy for the 26 sequence blocks was 96.63% $\pm$ 2.34% for the EXP group and 96.31% $\pm$ 2.29% for the NOEXP group. A repeated measures ANOVA of accuracy for TIMExAWARENESS showed no main effect of time ($F(1,15)=0.233$, $p=0.637$) or between conditions of awareness($F(1,15)=0.178$, $p=0.679$).

3.6.3 Behavioral Results: Latency

Baseline latency showed a significant difference for awareness (F(666,1)=7.938, p=0.005), with EXP subjects having a faster baseline mean latency (EXP=357.93 $\pm$ 85.35 msec, NOEXP=378.93 $\pm$ 100.52 msec). A repeated measures ANOVA of z score performance for TIMExAWARENESS demonstrated a main effect of time (F(25,415)=11.0280, p<0.001) and awareness (F(1,415)=311.6309, p<0.001) with an interaction effect of awareness (F(25,415)=3.9422, p<0.001). Final z scores showed a decrease of -4.055 $\pm$ 2.228 for the EXP group and -1.132 $\pm$ 0.619 for the NOEXP group. A simple main effects analysis showed statistically significant differences (p<0.05) in z score performance between groups appearing by Block 8. Representative individual performance graphs are shown in Fig 3.3. It was noted that behavior predictive of awareness varied between individuals, with (5) subjects demonstrating awareness before Block 20, labeled as Early Learners (see Fig 3.3a), and (4) subjects demonstrating awareness after Block 20, labeled as Late Learners (see Fig 3.3b). It was also noted that all subjects in the NOEXP group demonstrated an overall decrease in latency suggestive of implicit sequential learning (see Fig 3.3c).

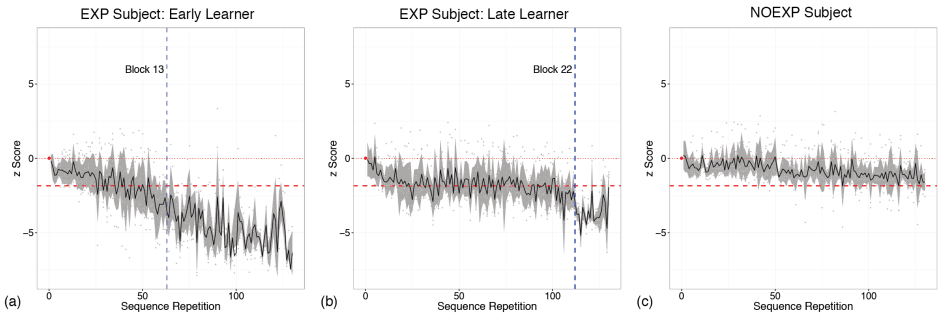


Figure 3.3: **Representative individual performance graphs for the aware (EXP) and unaware (NOEXP) groups.** The thick, dashed red line indicates each subject's individualized baseline-referenced threshold calculated at a z-score of -1.85. The vertical blue line (3a and 3b) indicate the block at which EXP subjects demonstrated two consecutive sequence repetitions with performance below threshold. Although Subject 16 shown in Figure 3.3(c) did not demonstrate behavior below threshold, there was a consistent decrease in latency over the experiment suggestive of implicit sequential learning.

3.6.4 EEG Results

EEG data for one subject was unable to be collected, so neural results were available for 20 subjects. Two of the subjects were falsely classified as NOEXP, and labeled as false negative (FN) and were excluded from further EEG statistical analyses, resulting in a final number of 18 subjects for EEG analysis, with (9) EXP and (9) NOEXP subjects. Figs 3.4-3.7 shows head map topographies, VEP traces and dipole localizations for the VEP time periods of interest over the course of the experiment. For simplicity of visualization, images demonstrate activity over 9 sections of time, with 3 blocks combined in each time section.

Early N1 component (100-140 ms):

Figure 3.4a shows the head map activity changes over the experiment and Figure 3.4b shows the Early N1 over frontal electrodes (F1, FZ, F2) for both EXP and NOEXP groups. A repeated measures ANOVA for TIMExAWARENESS effect on early N1 peak amplitude demonstrated a main effect of time ($F(8,64)=3.3795$, $p=0.011$) and awareness ($F(1,8)=5.3712$, $p=0.051$), with no interaction effect ($F(8,64)=1.3691$, $p=0.2745$). Post hoc analysis demonstrated the EXP group N1 amplitude was significantly greater ($p<0.05$) than the NOEXP group for Blocks 4-6, 7-9, and 10-12. The EXP group demonstrated a significant difference in amplitude, relative to baseline, by Blocks 4-6, while the NOEXP group did not demonstrate a significance difference from baseline until Blocks 13-15 (see Figure 3.4a). The EXP group demonstrated peak activations at Blocks 16-18, followed by a secondary peak at Blocks 25-27, showing similar clustering to the Early and Late Learner behaviors. Dipole localization results revealed a cluster localized to the anterior cingulate cortex (ACC) correlating with the early N1 component (see Figure 3.4c for dipole centroid image and Table 3.4 for Talairach coordinates and cluster distribution).

Late N1 component (140-170 ms):

Figure 3.5a shows the head maps demonstrating activity changes over time, while Figure 3.5b shows VEP changes over the parietal lobe electrodes (P1,P3,P2,P4). Repeated measures ANOVA

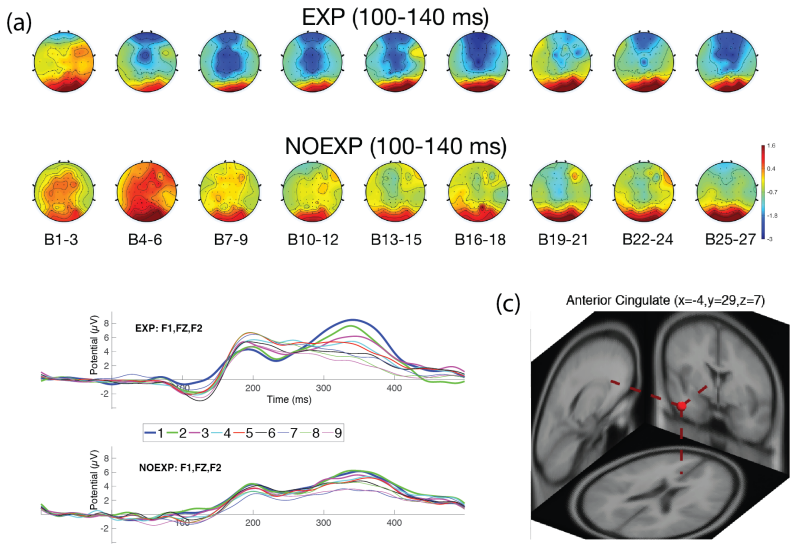


Figure 3.4: **Neural activity for early N1 over frontal region.** (a) Head maps for 9 periods of time during early N1 (100-140 msec). (b) VEP graph over frontal electrodes (F1,FZ,F2). (c) Dipole localization for early N1 component over anterior cingulate region.

for TIMExAWARENESS demonstrated no main effect of time ($F(8,64)=0.5811$, $p=0.817$), or awareness ($F(1,8)=1.0328$,$p=0.374$), with an interaction effect nearing significance ($F(8,64)=2.1245$, $p=0.077$). Post hoc analysis showed the EXP group had a significant difference in amplitude, relative to baseline, by Blocks 4-6, while the NOEXP group demonstrated a significant difference by Blocks 7-9. The NOEXP group maintained a relatively stable N1 for the remainder of the experiment, while the EXP group demonstrated a reduced N1 amplitude toward the end of the experiment, reaching levels similar to baseline by Blocks 19-21. ICA cluster analysis identified two clusters correlating with the late N1 component, with dipole localizations at the left and right precuneus (see Figure 3.5c, 3.5e for dipole centroid images and Table 3.4 for Talairach coordinates and cluster distribution). Cluster VEP's revealed a larger late N1 for the EXP group over the left precuneus, while the NOEXP group demonstrated a larger late N1 component over the right precuneus (see Figure 3.5d and 3.5f). Based on the VEP differences noted between the left and right parietal dipole clusters, an additional repeated measures ANOVA for TIMExAWARENESS was made for the right (P2 & P4) and left (P1 & P3) parietal electrodes separately. The right parietal electrodes showed no main effect of time ($F(8,64)=0.6419$, $p=0.739$), or awareness ($F(1,8)=0.1.1044$, $p=0.347$) and

no interaction effect (F(8,64)=1.5351, p=0.206). The left parietal electrodes also showed no main effect of time (F(8,64)=0.6639, p=0.769) or awareness (F(1,8)=0.9479,p=0.404). However, there was an interaction effect seen (F(8,64)=2.7751, p=0.024), with EXP subjects demonstrating a significant difference in amplitude, relative, to baseline, by Blocks 4-6, while the NOEXP group did not show a significant change in amplitude.

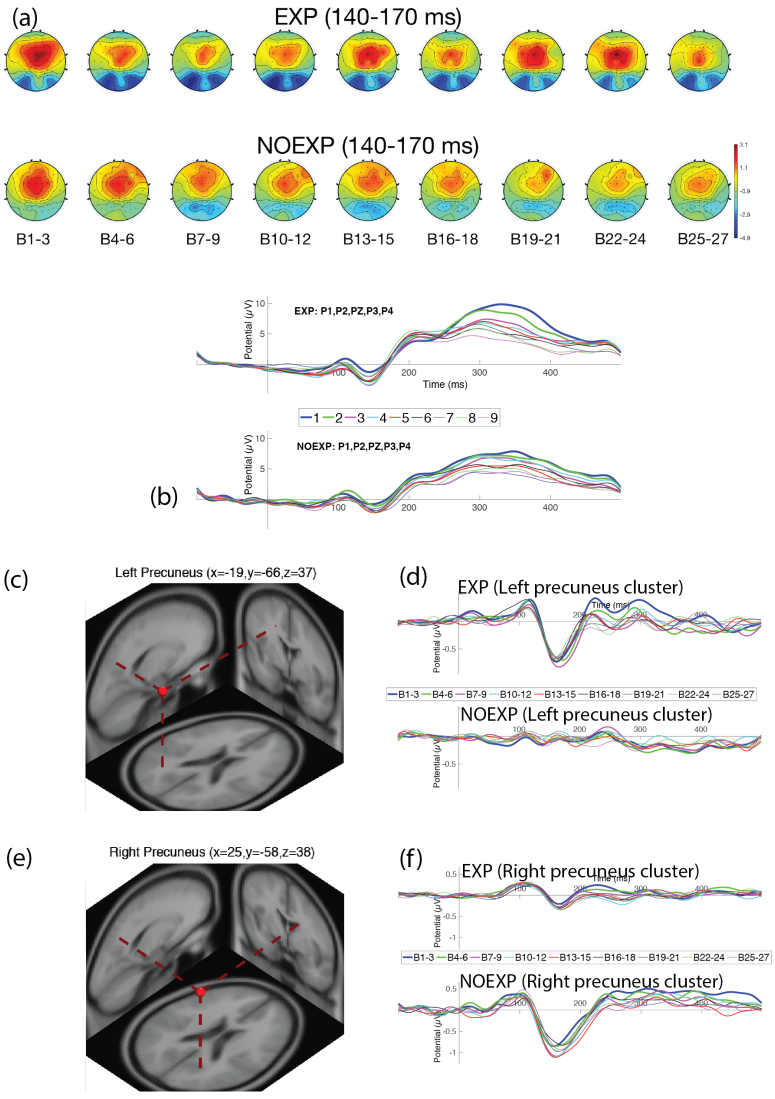


Figure 3.5: **Neural activity for late N1 over parietal region.** (a) Head maps for 9 periods of time during late N1 (140-170 msec). (b) VEP graph over parietal electrodes (P1,P2,P3,P4). (c) Dipole localization for late N1 component over left precuneus region. (d) VEP for left precuneus cluster. (e) Dipole localization for late N1 component over right precuneus region. (f) VEP for right precuneus cluster.

P2 component (180-210 ms):

Figure 3.6a shows head map changes for the P2 time period demonstrating the peak activity at Blocks 13-15 and 22-24. The P2 VEP component (see Figure 3.6b) showed no significant main effect of time ($F(8,64)=1.1908$, $p=0.392$) or awareness ($F(1,8)=1.6912$, $p=0.247$), but did reveal a significant interaction effect ($F(8,64)=3.7882$, $p=0.002$). Post hoc analysis revealed the EXP group had a significant difference in peak amplitude, relative to baseline compared to the NOEXP group. The largest significant differences were noted to occur at Blocks 13-15 and Blocks 22-24, again clustering in two time periods similar to the Early and Late Learner behaviors. The NOEXP group did not demonstrate a significant change from baseline over the course of the experiment. The ICA cluster correlating with the P2 component showed a dipole localization at the left supplementary motor area (SMA) as shown in Figure 3.6c (refer also to Table 3.4 for Talairach coordinates and cluster distribution).

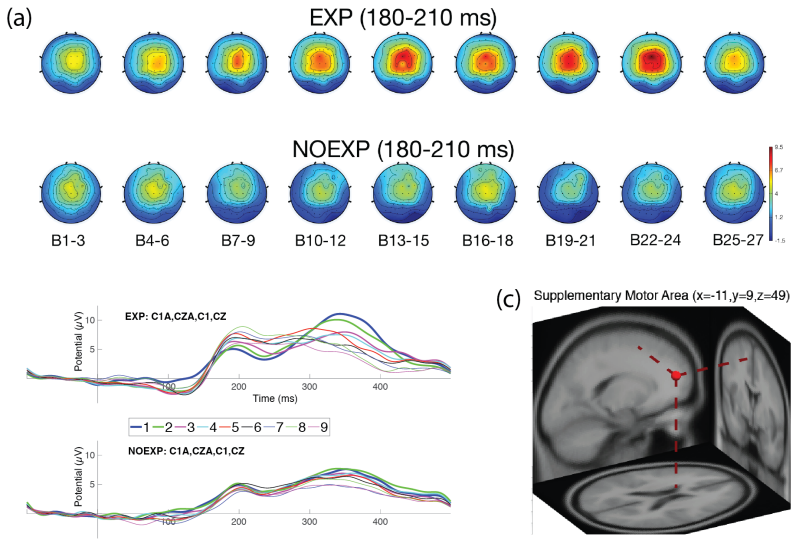


Figure 3.6: **Neural activity for P2 over left frontocentral region.** (a) Head maps for 9 periods of time during P2 (180-210 msec). (b) VEP graph over left frontocentral electrodes (C1A,CZA,C1,CZ). (c) Dipole localization for P2 component over left supplementary motor area.

P3 component (275-375 ms):

Head maps in Figure 3.7a show that both groups demonstrated a steady decline in peak amplitude over the course of the experiment. Peak amplitude for the P3 VEP component over centroparietal electrodes (see Figure 3.7b) revealed a significant main effect of time ($F(8,64)=8.6358$, $p=0.0035$), but not for awareness ($F(1,8)=0.2052$, $p=0.698$), with no interaction effect ($F(8,64)=1.1135$, $p=0.405$). Post hoc, pairwise comparison, revealed a significant difference ($p<0.05$), from baseline, by Blocks 13-15 for the EXP group and by Blocks 19-21 for the NOEXP group. Two clusters correlating with the P300 component showed dipole localization at the paracentral region (see Figure 3.7c) and the cingulate cortex (see Figure 3.7d). Separation of dipole localizations, by awareness, indicated localization for EXP subjects at precuneus/cingulate cortices, and paracentral/cingulate localizations for NOEXP subjects. See Table 3.4 for Talairach coordinates and cluster distribution).

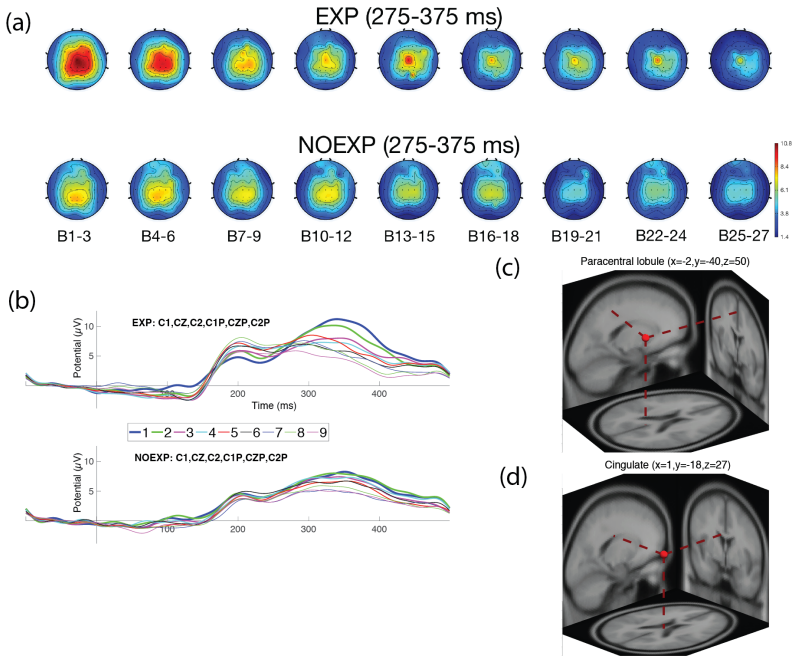


Figure 3.7: **Neural activity for P3 over centroparietal region.** (a) Head maps for 9 periods of time during P3 (275-375 msec). (b) VEP graph over centroparietal electrodes (C1,CZ, C2, C1P, CPZ, C2P). (c) Dipole localization for P3 component over paracentral region and (d) cingulate cortex.

Table 3.4: **Talairach coordinates and clustering values for source localization**

VEP Component	Dipole Localization	Talairach coordinates (x,y,z)	Residual variance	Percent of subjects included in cluster	EXP/NOEXP representation in cluster
$EarlyN1$	Anterior cingulate (BA 24)	(-4, 29, 7)	7.82%	38.9%	(4) EXP (3) NOEXP
$LateN1$	Right precuneus (BA 7)	(25,-58,38)	5.12%	72.2%	(8) EXP (5) NOEXP
$LateN1$	Left precuneus (BA 7)	(-19, -66, 37)	4.58%	66.7%	(5) EXP (7) NOEXP
$P2$	Medial frontal gyrus (BA 6)	(-11, 9, 49)	7.18%	88.9%	(9) EXP (7) NOEXP
$P3$	Paracentral lobule (BA 5)	(0, -41, 52)	7.68%	44.4%	(2) EXP (6) NOEXP
$P3$	Precuneus (BA 7)	(-3, -46, 47)	7.52%	44.4%	(4) EXP (4) NOEXP
$P3$	Cingulate (BA 23)	(1, -18, 27)	8.00%	61.1%	(8) EXP (4) NOEXP

3.6.5 Neurobehavioral correlation analysis between timing of EXP behavior and peak VEP component amplitude

Correlational analysis between timing of individual behavior (drop below threshold for EXP and lowest z-score for NOEXP) and peak VEP component amplitude activity was examined over the course of all 27 blocks. Neurobehavioral correlational results for each VEP component, with Spearman correlation rho values, are summarized in Table 3.5. For the early N1, there was a significant correlation for the EXP group (rho=0.68, p=0.041), but not for the NOEXP group (rho=-0.16, p=0.684). There was no significant correlation for either group with the late N1 component over right parietal (EXP: rho=-0.09, p=0.811 and NOEXP: rho=0.29, p=0.448). The left parietal correlation results revealed a significant correlation for EXP subjects (rho=0.82, p=0.007), but not for NOEXP subjects (rho=-0.33, p=0.378). The P2 also demonstrated a strong correlation for the EXP group (rho=0.82, p=0.006), with no significant correlation for the NOEXP group (rho=-0.39, p=0.298). The P3 component over centroparietal electrodes, and posterior cingulate

dipole localization, did not reveal significant correlations for either group, although EXP subject correlation was nearing significance (EXP: rho=0.62, p=0.076 and NOEXP: rho=-0.14, p=0.726).

Table 3.5: **Spearman significance values for neurobehavioral correlations.**

	Early N1:ACC		**Late N1: Left Parietal**		**Late N1: Right Parietal**		**P2:SMA**		**P3:PCC**	
Group	EXP*	NOEXP	EXP**	NOEXP	EXP	NOEXP	EXP**	NOEXP	EXP†	NOEXP
Rho	0.69	-0.16	0.82	-0.33	-0.09	0.29	0.82	-0.39	0.62	-0.14
pvalue	0.041	0.684	0.007	0.378	0.811	0.448	0.006	0.298	0.076	0.726

†p<0.1; *p<0.05; **p<0.01

3.6.6 Neurobehavioral Correlation Analysis: Change in latency over time relative to change in P3 amplitude over time

Due to the localization of the P3 over sensory association and cingulate regions, along with the common overall decrease in amplitude noted for both EXP and NOEXP groups, it was hypothesized that the P3 may represent adaptation to the paradigm not specific to the presence awareness. As both the P3 amplitude and sequence response latency decreased for all subjects over the course of the experiment, an additional correlational analysis was conducted between the P3 amplitude and subject mean response latency for each block to further explore the neurobehavioral relevance of the significant change in P3 amplitude seen in both groups. A mixed model analysis with fixed effects for latency and awareness, and random effects of subject intercept and slope revealed a significant relationship of P3 amplitude and latency (t(442)=2.719, p=0.0068) and a significant main effect of awareness (t(16)=2.660, p=0.0171) with no interaction effect. EXP subjects had a greater mean intercept (335.47 $\pm$ 94.31 uV^2) compared to NOEXP subjects (84.64 $\pm$ 136.15 uV^2). Figure 3.8 shows two representative individual correlation plots, one from each group.

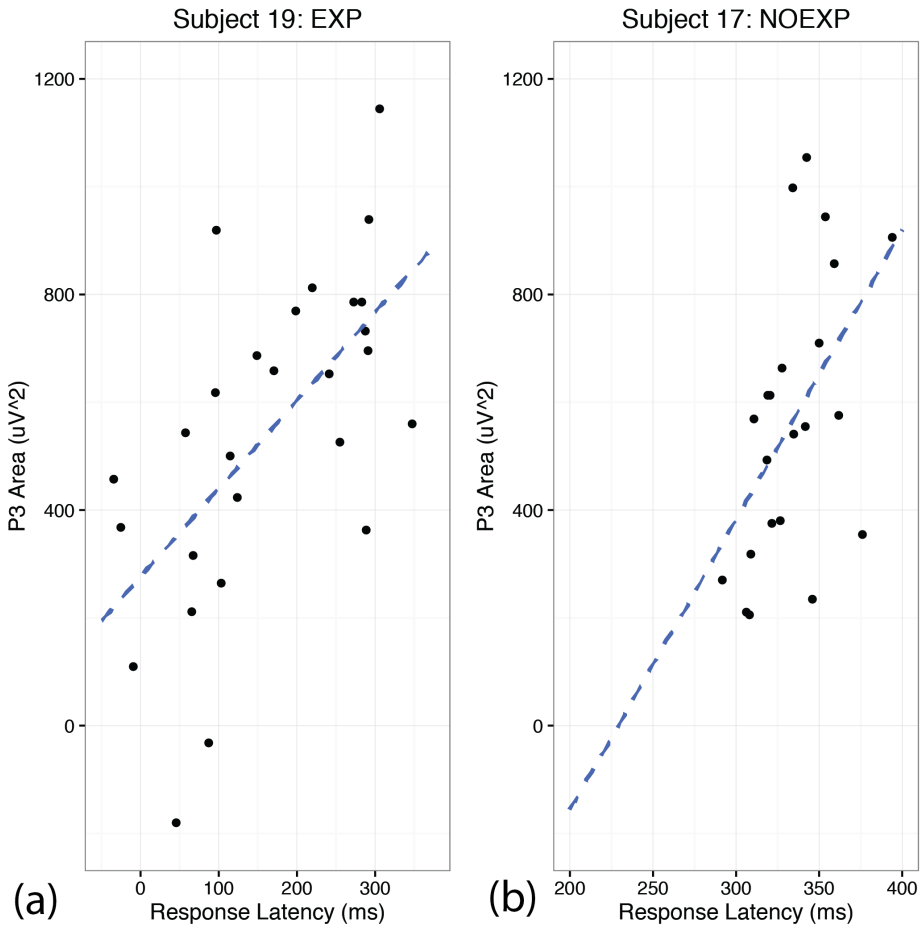


Figure 3.8: **Representative individual correlation plots of P3 amplitude with latency.** Correlation of P300 amplitude with response latency for each block. Note the strong correlation regardless of awareness level.

3.6.7 Neural correlation analysis between timing of peak VEP component amplitude

As all VEP components demonstrated significant, or near significant, correlations with EXP behavior, an additional correlational analysis was conducted for the timing of each VEP component. Neural correlations between VEP components in regions demonstrating significant correlation with behavior demonstrated significant correlations between the early N1, left late N1, P2 and posterior cingulate P3 for EXP subjects that was not observed for NOEXP subjects. Spearman correlation values are summarized in Table 3.6. Timing of the peak occurrences for these 4 regions in EXP subjects is plotted in Figure 3.9. A mixed model analysis for timing of peak amplitude occurrence with fixed effect of VEP component order (P3, Late N1, P2 and Early N1) and random effect

of subject revealed a significant difference in the timing of the P3 peak amplitude (t(48)=6.98, p<0.001) with EXP subjects demonstrating the peak P3 component 3 blocks earlier (EXP: Block 9.55 $\pm$ 6.00, NOEXP: Block 12.55 $\pm$ 4.24). There was no significant relationship between timing of Late N2, P2 or Early N1 components and P3 peak timing (LateN1: t(48)=1.24, p=0.221, P2: t(48)=1.00, p=0.321, EarlyN1: t(48)=0.295, p=0.769). Adding the fixed effect of awareness did not significantly improve the model (X^2(7,1) = 0.911, p=0.339), but the addition of an interaction effect resulted in significant model improvement (X^2(10,3)=16.14, p=0.002). Due to the interaction effect, a separate repeated measures analysis was conducted for EXP and NOEXP subjects, utilizing Tukey contrasts for multiple comparison of means. The NOEXP subjects demonstrated no significant linear trends between VEP component peak timing. The EXP group, however, demonstrated a linear trend between P3, LateN1 and P2, leveling off at EarlyN1 as shown in Table 3.6 and Figure 3.9.

Table 3.6: **Correlation matrix for EXP and NOEXP groups shown in Figure 3.9.**

Linear Hypotheses	**EXP**	**NOEXP**
$LateN1 - P3 = 0$	p=0.007	p=0.747
$P2 - P3 = 0$	p<0.001	p=0.140
$EarlyN1 - P3 = 0$	p<0.001	p=0.994
$P2 - LateN1 = 0$	p=0.007	p=0.998
$EarlyN1 - LateN1 = 0$	p<0.001	p=0.913
$EarlyN1 - P2 = 0$	p=0.860	p=0.914

3.7 Discussion

The overarching goal of Aim 1 was to identify and validate a reliable behavioral predictor for the presence of incidental explicit awareness in a sequential motor task. This type of incidental discovery may be reflective of the neuronal interactions occurring during the exploratory motor learning often utilized in rehabilitation. Recent studies examining such incidentally developed explicit awareness show benefits of enhanced perceptual sensitivity and increased motivational vigor [82, 31], but have not attempted to identify when such awareness occurred. A major challenge for such indicators is detecting the presence of awareness without interfering with the incidental nature

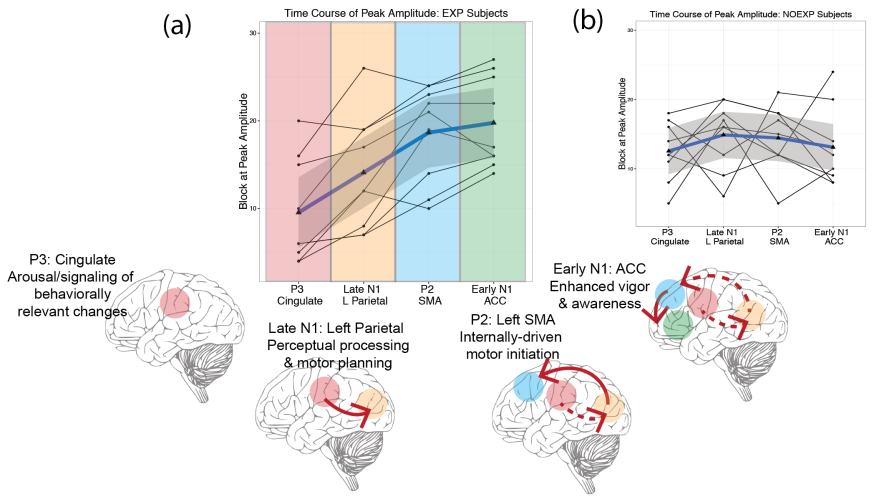


Figure 3.9: **Timing of peak VEP component occurrences.** (a) EXP timing with proposed timing of peak activations required for development of incidental explicit awareness. (b) NOEXP timing. Note the seemingly random progression of peak activation between subjects.

of the discovery. Results from Experiment 1, provided an individualized indicator, with neurobehavioral validation in Experiment 2. Our work showed frontoparietal activations in EXP subjects, demonstrating a facilitative, learning-dependent interaction between perceptual and motor regions specific to explicit sequential motor learning.

3.7.1 Why the model works

The proposed behavioral indicator utilizes the dramatic decrease in latency observed for subjects expressing explicit awareness [28, 83]. Previous studies examining incidental awareness predictors utilized tasks focused on single event occurrences rather than full sequence awareness [28]. Our indicator addresses this concern by examining behavior over each sequence repetition, identifying when a subject is aware of the entire sequence. Utilizing baseline performance accounts for initial variability in visuomotor performance, allowing for an individualized approach in awareness identification [75]. The identified threshold indicator of two consecutive sequence performances below a relative z-score of -1.85 from Experiment 1, showed 81.8% sensitivity and 100% specificity for subjects in Experiment 2.

3.7.2 Temporal course of neural activations with explicit awareness

Peak amplitude analysis of VEP components demonstrates a consistent temporal course of neural activity, suggesting involvement of parieto-frontal areas in perceptual processing and subsequent motor execution of the visuomotor task. Results showed significant correlations between timing of predicted EXP awareness and peak amplitude timing over cingulate, left parietal, premotor and prefrontal regions. The sequential peak activation of these areas suggest a facilitative neural network associated with development of incidental explicit awareness in sequential motor learning. Such learning-dependent changes have been noted with visual and somatosensory perceptual learning [213, 214], but have not examined the impact of such learning on motor tasks. Studies utilizing motor tasks typically assess learning-dependent changes over multiple sessions [215, 216, 217]. Results from this study examined perceptual and motor related learning-dependent changes within a single session, providing insight into changes potentially occurring during a single rehabilitative session.

EXP subjects showed a strong linear trend for the timing of peak activations in a frontoparietal network, progressing from posterior cingulate (PCC) to left precuneus (LPcun), left supplementary motor area (SMA) and anterior cingulate (ACC). The presence of peak activations in VEP components may be indicative of optimized neuronal communication through mechanisms such as enhanced synaptic efficiency reflective of Hebbian learning [213] or altered communication patterns increasing task-relevant neuronal activity and reducing activity in task-irrelevant neurons [204]. This learning may involve similar mechanisms as proposed for the development of predictive mirror neurons which utilize spike-timing-dependent plasticity and are suggested to require both consistently causal and contingent neuronal interactions [218]. Causality requires consistent presynaptic firing within a time window on the order of tens of milliseconds [219], potentially reflected in the progression of the amplitude of individual VEP components. For learning to occur, however, causality must also be accompanied by contingency, the determination of whether the presynaptic activity is informative about postsynaptic activity, and functions under a time window of minutes [220]. The VEP components associated with each region indicates differ-

ential timing and separate roles in the processing and decision-making aspects of integrating the stimulus-response mapping, providing both a causal and contingent interaction between perceptual processing and motor actions. As proposed below, this consistent causality and contingency may provide the mechanism allowing a person to shift from a reliance on external stimuli for movement execution to an internally-generated learned motor sequence.

The first peak activation for EXP subjects, occurred with the P3 which localized to the PCC. The P3 is attributed with engagement of attention and novelty processing [198], while the PCC is involved in attentional arousal and signaling of behaviorally relevant changes in stimuli [221], and the precuneus with perceptual decision-making [114]. The accumulation of visual and somatosensory evidence over time may result in enhanced synaptic communication within cingulate neuronal pools, indicative of the neural recognition of a behaviorally relevant regularity in the environment. Reaching peak activation may alter attention over bilateral precuneus regions to include an external focus on visual stimuli, and an internal focus to assist in integrating visuospatial information with motor execution.

The inclusion of an internal focus is reflected in the subsequent peak activation of the late N1, over the LPcun region. This component is attributed to perceptual processing of stimuli [190], often localizing over posterior parietal cortex, including the precuneus. Functionally, the precuneus has been shown to demonstrate a hemispheric specialization with visuomotor tasks. The right precuneus (RPcun) is attributed to stimulus encoding and visuospatial transformations necessary for eye-hand coordinated movements [115, 116, 114], while LPcun activation appears to be involved with praxis planning and encoding of future intentions [120, 118, 121]. The enhanced LPcun activation observed for EXP subjects provides evidence for an attentional resource shift to include the left parietal region which may allow for sensorimotor integration of visual stimuli information with finger movement dynamics leading to development of an internal representation of the visuomotor relationship [222]. This visuomotor relationship may be used as a predictive motor plan which can assist in accumulating probabilistic data to test the possible regularity in visual stimuli or motor responses signaled by the PCC. Unlike the LPcun, the RPcun did not show a significant correlation

with behavior, suggesting that subjects continued to perform visuospatial transformations regardless of awareness. Although, as explained below, the function of such visuomotor transformations may shift as awareness develops.

After left parietal areas demonstrate peak activity, there is a concurrent peak activation over left SMA and ACC regions, in the P2 and early N1 components, respectively. The P2 is known to be sensitive to tasks comparing sensory inputs and presence of conscious awareness [200, 223, 211, 224], while the SMA has long been associated with internally-guided movements [210]. The progressive increase in SMA activity, therefore, may reflect enhanced attentional resources for initiation of movement patterns less dependent on the external stimulus. While both EXP and NOEXP subjects demonstrated dipole activation over the SMA, EXP subjects showed a much greater increase in amplitude, with peak occurrence being highly correlated with explicit behavior. This correlation provides evidence for a shift in EXP subjects to the use of internally generated movements, accompanied by a conscious awareness of these movements.

Peak activation over ACC regions during the early N1 timeframe may reflect a neural representation of enhanced attentional resources involved in explicitly executing the sequence [20] and the enhanced motivation observed with explicit awareness [31]. The early N1 component is typically associated with attentional gating [192, 185], while the ACC is attributed to multiple cognitive roles such as decision-making, reward, learning and consciousness [225]. The occurrence of peak activity for the ACC and SMA regions suggest reciprocal communication between anticipatory visuospatial confirmation of the visual cue and facilitation of predicted motor behavior as conscious awareness of the sequence develops.

3.7.3 Neural activations without explicit awareness

NOEXP subjects did not show any significant correlations in peak activity. This group demonstrated an irregular sequence of regional peak activation between subjects, indicating a decoupling in peak timing between the cingulate arousal, perceptual integration of stimulus-response and internalization of motor execution. The lower amplitude of LPcun late N1 activity, suggest that NOEXP

subjects fail to make the attentional shift, resulting in a retained reliance on the external visual cues for motor initiation. Without the transition to enhanced left parietal activity, NOEXP subjects may have been unable to form an internal representation of the sequence pattern, preventing the shift to internal movement execution and development of conscious awareness of movement patterns.

3.8 Conclusions

The results of Aim 1 provide a validated, indirect, individualized, behavioral indicator for detecting both the presence, and timing of, the development of explicit awareness in a sequential motor task. The individual nature of the indicator provides a tool for monitoring progress in rehabilitative, exploratory motor learning paradigms. Utilization of the proposed indicator with EEG recordings provided neurobehavioral evidence of a learning-dependent, facilitative fronto-parietal network involved with the development of explicit awareness in a sequential motor task. This incidentally-driven facilitative network may provide valuable lines of research for rehabilitative programs using exploratory learning.

The fronto-parietal network learning-dependent changes observed is proposed to facilitate changes in perceptual processing which enhance motor learning. What remains unclear, however, is whether these learning-dependent changes are not occurring in NOEXP subjects, or if they did not have enough time for facilitative neuronal interactions needed for awareness to occur. Furthermore, as the current study focused on exposure to a single sequence, it is unclear the impact of the perceptual processing changes, associated with awareness, on the ability to transfer such learning to novel sequence patterns. Multiple studies have shown the presence of explicit awareness to be detrimental to generalization, but have done so utilizing an intentional paradigm, informing subjects of the presence of a sequence. The next chapter utilizes the validated individualized indicator from Aim 1, as a method in which to explore factors correlative with subjects failing to develop awareness and the impact of incidentally developed awareness on generalization.

Additionally, the apparent pivotal role of enhanced cingulate activity may help explain sequential learning detriments seen with amputees, stroke patients and with aging [12, 4, 5]. The

utilization of reaction time as the sole behavioral measure in Aim 1 limits the ability to disentangle the contributions of the visual and motor systems in development of awareness. Recent studies by Moisello et al. have demonstrated that awareness is accompanied not only by changes in overall reaction time, but also changes in movement time and movement patterns [17, 74]. Both studies present data that suggests the value of anticipatory movement onset with awareness may be to provide additional time for more precise movements. To further elucidate the motoric significance of the SMA peak, Chapter 5 will examine correlations of the SMA peak with additional behavioral measures such as movement onset, movement time, and kinematic variables such as movement peak velocity, smoothness and directional error. These parameters are of particular importance in rehabilitative settings examining motor learning and motor control. How the peak activity over LPcun regions contribute to awareness development will be further explored through utilization of eye-tracking to identify anticipatory eye movements. Exploration into these questions will additionally be addressed in Chapter 5.

CHAPTER 4
SPECIFIC AIM 2

4.1 Introduction

The purpose of Aim 2 was to evaluate the effect of incidentally developed explicit awareness on the transfer to a novel, more difficult motor sequence. A critical component of motor skill acquisition is the ability to transfer motor learning from one context to a novel, or more complex scenario. The ability to generalize, or transfer skills, has been shown to be affected by the way in which the initial motor skill is encoded [80], with differential mechanisms being utilized for implicit and explicit learning [79, 54]. Additionally, it has been shown that consolidative processes, which serve to strengthen memory traces, may reduce the impact of interference and facilitate transfer [226, 54]. As described previously in Chapter 1, multiple studies have demonstrated that the presence of intentionally developed explicit awareness during motor learning interferes with transfer to a novel task during a within training session, but have done so utilizing an intentional paradigm, with subjects being aware that a sequence is present [23, 79, 22]. The effects of incidentally developed awareness on generalization are less understood. To examine the effect of incidentally developed awareness requires the ability to indirectly detect if a person has developed awareness. The results from Aim 1 provided such an indicator, introducing the opportunity to compare neurobehavioral differences between explicit and non-explicit subjects in their ability to transfer to a novel, more complex sequence.

As in Aim 1, the issue of individual variation in learning rates presents a potential confound when examining neurobehavioral changes from a group perspective [92]. The indicator proposed in Aim 1, provides an opportunity to examine neural changes based on individual timing of explicit awareness and the correlated neural changes associated with such awareness. This individualized approach may reveal common neural activations for those people developing awareness and those

failing to achieve awareness or transfer awareness skills to a novel situation. Understanding the neurobehavioral factors contributing to the successful learning and generalization of a motor task, from an individualized perspective, may provide insight into why some people are more effective at learning a motor task and what limiting factors may exist for non-learning individuals.

To this end, Aim 2 utilized the individualized threshold indicator as a tool to classify subjects as EXP or NOEXP during a priming, 7-key sequence, to examine the effect of awareness on the ability to transfer to a novel, more complex 10-key sequence. To address the potential confound of changes associated with practice, a control group was also included for comparison. As with Aim 1, a two-experiment approach was utilized. Experiment 1 examined the behavioral effects of awareness on generalization, with a focus on speed and accuracy of both the priming and transfer sequence. Experiment 2, examined the neurobehavioral correlates associated with awareness development, with a goal of identifying predictors of awareness and successful transfer of the learned motor skill.

4.2 Hypotheses

Experiment 1: Behavioral Effects of Explicit Awareness on Generalization. While previous studies have demonstrated the detrimental effects of explicit awareness on the ability to generalize to novel tasks, they have done so utilizing an intentional paradigm [23, 227, 80]. As discussed above, this may result from competition due to the recruitment of both the implicit and explicit systems for movement optimization from the beginning of the experiment. However, the incidental development of awareness may utilize the facilitative network identified in Aim 1 [228], allowing for optimization from the implicit system to occur before recruitment of the explicit system. Therefore, it was hypothesized that subjects developing explicit awareness in an incidental paradigm will demonstrate better transfer to a novel, more complex sequence than those subjects failing to develop awareness.

Experiment 2: Neurobehavioral Correlates Associated with Explicit Awareness and Subsequent Transfer. Multiple studies have demonstrated a role of working memory in the ability to

acquire sequential motor skills [229, 201, 230], therefore it was hypothesized that subjects demonstrating reduced working memory will fail to develop awareness. Additionally, the recruitment of a facilitative network utilized in the incidental development of explicit awareness during motor learning revealed from Aim 1 [228] was hypothesized to be present for subjects classified as explicit during the priming sequence. Individuals recruiting this network are hypothesized to demonstrate neural activation reflective of continued recruitment of the facilitative network to promote discovery of the novel, transfer sequence.

4.3 Materials and Methods

4.3.1 Experiment 1: Behavioral Effects of Explicit Awareness on Generalization

Subjects:

Twenty-three right-handed (age 18-27 years; 12 females), neurologically healthy adults were recruited to participate in the study. All subjects provided written, informed consent, and the Georgia Institute of Technology Institutional Review Board approved all methods. Subjects completed an Edinburgh Handedness Inventory [205] to assess the level of handedness along with a short questionnaire regarding any previous musical training they had received. Only subjects with a handedness score greater than 0.6 (indicating right hand dominance), and less than 3 years formal musical training, were included in the study.

Experimental Paradigm

Subjects were randomly placed in one of two groups, a priming group (PRIMING) or a control group (CONTROL). As the PRIMING group would later be classified into two additional groups, explicitly aware of the priming sequence (EXP) and not explicitly aware (NOEXP), twice as many subjects were assigned to the PRIMING group (16 subjects) as the CONTROL group (7 subjects). The same 7-element and 10-element sequences from Specific Aim 1 were used, with the 7-element sequence serving as the priming sequence and the 10-element sequence serving as the

transfer sequence. Stimuli presentation was done utilizing the same MATLAB presentation code as was utilized for Experiment 2 of Specific Aim 1. All subjects experienced a 50-element random, baseline sequence in the first block which was utilized to calculate each subject's individualized threshold for awareness classification. Individual thresholds were calculated as the latency which represents a -1.85 z-score of baseline performance (See Eq 3.1). Specifics of the experimental paradigm for the priming and control groups is described in more detail below.

PRIMING Group: Following the baseline block, subjects in the PRIMING group experienced 20 blocks consisting of (7) repetitions of a 7-element sequence. This provided a total of 140 sequence repetitions of the priming sequence. The use of 20 blocks was based on results from Specific Aim 1, demonstrating that this was sufficient to result in some, but not all, subjects developing awareness. Based on the sensitivity and specificity results from Specific Aim 1, subjects demonstrating two (2) consecutive sequence repetitions with upper confidence interval latency performance below the individualized threshold were classified as explicit (EXP) for the priming sequence. Subjects whose behavior stayed above the calculated threshold, were classified as NOEXP on the priming sequence.

Upon completion of the priming sequence, participants were presented with a second 50-key random sequence. Latency performance on the second random sequence was utilized to calculate a new threshold. This provided an adjusted threshold to address any behavioral changes associated with practice effects from the priming sequence. Upon completing the second random sequence, subjects were given a 1-minute rest period, with a screen message informing them they were moving to the second part of the experiment. This message was utilized to both provide motivation for the priming group and to inform subjects of a change in the experiment. After the rest period, subjects were presented the transfer, 10-element sequence. Subjects experienced five (5) repetitions of the 10-element sequence per block, with a 10-second rest between blocks. The number of blocks a subject experienced was determined by their latency performance in each block. If a subject demonstrated performance of two (2) consecutive sequence repetitions with an upper confidence interval latency below the calculated threshold, they experienced one more block and then

exited the transfer sequence. Subjects not dropping below threshold within 30 blocks were exited from the transfer sequence, providing a maximum of 150 sequence repetitions of the transfer sequence. Immediately upon exiting the transfer sequence, subjects were asked if they noticed a pattern within the presentation. If they answered yes, subjects were asked to replicate the pattern noticed. If they answered no, subjects were informed there was a pattern present and asked to replicate the pattern to the best of their ability. Replication responses were recorded to assess for accuracy. Subjects able to repeat the sequence with 100% accuracy were identified as explicitly aware on the 10-element sequence, EXP. As some subjects may learn the sequence at a different starting point than what was presented, accuracy was not limited to the presented starting point. For example, a subject experiencing the 10-element sequence 4-2-1-4-3-1-2-4-1-3, would be classified as EXP if their recall was full recall in order (4-2-1-4-3-1-2-4-1-3), or if it was full recall, but starting somewhere in the middle of the sequence (e.g, starting with the 4th element of the actual sequence 4-3-1-2-4-1-3-4-2-1). Subjects unable to repeat the sequence with 100% accuracy were categorized as not explicitly aware, NOEXP. Due to the exposure to two different sequences, subjects in the PRIMING group could be classified into four awareness categories: (1) explicit behavior on the priming sequence and explicit recall on the transfer sequence (EXP), (2) no explicit behavior on the priming sequence and non-explicit recall on the transfer sequence (NOEXP), (3) explicit behavior on the priming sequence, but non-explicit recall on the transfer sequence (EXP_NOEXP), and (4) non-explicit behavior on the priming sequence, but explicit recall on the transfer sequence (NOEXP_EXP). Experimental protocol is summarized in Figure 4.1.

CONTROL Group: The CONTROL group differed from the PRIMING group in the lack of exposure to the 7-element priming sequence. Therefore, CONTROL subjects experienced the first random block, immediately followed by a second random block. Performance on the second random block was used to calculate the individual threshold latencies for each CONTROL subject. Upon completion of the second random block, CONTROL subjects experienced the 10-element transfer sequence in the same manner as the PRIMING group, including the recall questions. CONTROL subjects could be classified as explicit or non-explicit in terms of recall, potentially

providing two categories CONTROL_EXP and CONTROL_NOEXP, respectively.

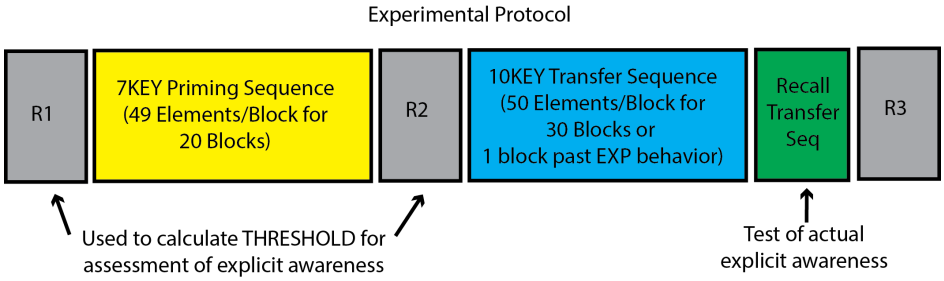


Figure 4.1: **Experimental Protocol.** Priming group subjects experienced both the priming sequence and transfer sequence. Control group subjects experienced the transfer sequence only.

Subjects:

4.3.2 Experiment 2: Neurobehavioral Effects of Explicit Awareness on Generalization

An additional twenty-eight right-handed (age 18-24 years; 12 females), neurologically healthy adults were recruited to participate in Experiment 2. All subjects provided written, informed consent, and the Georgia Institute of Technology Institutional Review Board approved all methods. As in Experiment 1, subjects completed an Edinburgh Handedness Inventory [205] to assess the level of handedness along with a short questionnaire regarding any previous musical training they had received. Only subjects with a handedness score greater than 0.6 (indicating right hand dominance), and less than 3 years formal musical training, were included in the study.

SRTT Behavioral Task

Experiment 2 utilized the same SRT behavioral task as introduced in Experiment 1. Changes to the presentation MATLAB code added a small black box appearing in the upper and lower left corners of the screen to allow for a photodiode impulse to be detected by StimTracker™ (Cedrus Corporation, San Pedro, CA) for subsequent EEG analysis. These boxes were covered to prevent visual distractions. A pulse was transmitted both at the onset of each stimulus and at the beginning of each block. Additionally, all subjects completed a 2-back working memory assessment (http://cognitivefun.net/test/4) at the beginning of the experiment. Subsequent to the

2-back task, subjects completed a demographic questionnaire and EEG preparation was conducted. This provided a minimum of 30 minutes between the 2-back task and the SRT task.

EEG Recording and Pre-Processing

EEG analysis utilized a 58-channel EEG cap (Electrocap, Eaton, OH) that recorded scalp potential activity (1000 Hz sampling rate, filtered at DC-100 Hz) via the SynAmps II^{TM} data acquisition system (Compumedics Neuroscan, Charlotte, NC). Data were referenced to the ear electrode and impedances kept below 5 k. The raw, continuous EEG data was then imported into MATLAB's EEGLAB [203] for pre-processing and analysis. Data was first band-pass filtered from 0.5-50 Hz. Data was then epoched from 100 ms pre- stimulus onset to 600 ms post-stimulus for all stimuli presentations with baseline correction from 100 to 0 msec, for subsequent event-related potential (VEP) analysis. An independent component analysis (ICA) was conducted utilizing EEGLAB's *runica* algorithm, to assist in removal of blink and other stereotypical movement artifact components. Selection of components for removal was based on visual inspection of scalp map localization, unusual spectral frequency patterns and irregular VEP-image activity. An average of 9 of 58 components per participant was selected for subtraction. After removal of artifact components, data was segmented into separate datasets, one for each block, Random 1 and Random 2 as well as all blocks completed for both the priming and transfer sequence. A separate dipole fitting analysis was then conducted, utilizing EEGLAB's DIPFIT plugin [180], for every block for each subject. Dipole localization was determined utilizing the Talairach Client application [208]. After pre-processing, all datasets were loaded into a STUDY structure for both individual and group analysis.

EEG Event-related Potential Area Amplitude Calculation

Individual subject datasets were loaded into EEGLAB Study design with identification of within subject condition of time (The priming sequence condition of time included Blocks 1-20 along with Random 1 and 2. The transfer sequence condition of time included all completed blocks,

along with Random 2 and 3.) VEP components of interest, and regions of interest, were based on our previous work (cite) which demonstrated a facilitative frontoparietal network in perceptual processing associated with explicit awareness. Due to individual changes in the VEP latencies observed, the VEP components post-stimulus time periods analyzed were expanded from those used in Specific Aim 1: (1) early N1: 80-130 ms, (2) late N1: 130-180 ms, (3) P2: 130-210 ms, and P3: 250-400 ms. The regions of interest for each component included: (1) early N1 over frontal electrodes (F1, FZ, F2) to examine attentional gating modulation, (2) late N1 over left and right parietal electrodes (P1,P3) and (P2,P4) to examine perceptual processing changes, (3) P2 over left frontocentral electrodes due to the right-handed nature of the task (C1, CZ, C1A, CZA) to examine changes related to sensorimotor comparison activity, and (4) P3 over centroparietal electrodes (C1, CZ, C2, C1P, PZA, C2P). Dipole localizations were conducted to verify similar localizations as were found in Specific Aim 1, demonstrating the source of the early N1 to be the anterior cingulate (ACC), the late N1 signal as the left and right precuneus regions, the P2 as the supplementary motor area (SMA), and the P3 as the posterior cingulate (PCC). VEP component amplitude values for each block were determined utilizing the signed area amplitude method as used in Specific Aim 1 [178].

Statistical Analyses of Behavioral Data

Two of the 51 subjects were falsely classified as EXP on the transfer sequence and were, therefore, removed from analysis. Due to only one subject being placed in the NOEXP_EXP group, this awareness group was not included in any subsequent statistical analyses, resulting in (4) awareness classification levels for analysis. All statistical comparisons were conducted utilizing factors of both condition (2 levels: PRIMING and CONTROL) and awareness classifications (4 levels: EXP, NOEXP, EXP_NOEXP, and CONTROL). Awareness classifications were determined by each individual's performance relative to the threshold for both the priming sequence and the transfer sequence.

Individual subject data was analyzed utilizing a custom MATLAB (Natick, MA) program. Sub-

ject data was first separated into both block and sequence repetitions. Individual average accuracy and z-scores, for each sequence repetition, was calculated and utilized for group comparisons. Z-scores for each response was calculated based on individual thresholds, as calculated from the random baseline immediately preceding the priming or transfer sequence. Due to the potential for unequal number of subjects to be in the various awareness classification groups, a repeated measures mixed model analysis was performed for all comparisons of accuracy, latency, z-score changes. When model comparisons indicated a main effect or interaction effect, pairwise comparisons were conducted with single-step Bonferonni corrections for multiple comparisons. The complexity of the models utilized is summarized below. Model comparisons utilized the AIC (Akaike information criterion) value, to assess and compare the relative quality of the proposed models. Models with the increased number of explanatory variables may explain data variability, but their complexity often sacrifice generalizability. AIC values provide a measure for the model which best accounts for variability with minimal complexity. Adjusted significance levels are reported at $p < 0.05$ for both model and pairwise comparisons.

Model 0 (M0) = Repeated measures (RM) with random effect of subject

Model 1 (M1) = RM with fixed effect of time (3 levels: Random 1,2,3)

Model 2 (M2) = RM with fixed effect of group (PRIMING: 2 levels or AWARENESS: 4 levels)

Model 3 (M3) = RM with interaction between fixed effects of time and group (3x2 and 3x4)

Statistical Analysis of Neurobehavioral Correlations

Neurobehavioral correlations were conducted between each subject's z score performance and the associated VEP components over the course of the priming and transfer sequence experiments. The behavioral performance variable, or block of interest (BOI), for EXP subjects, was the block at which behavior dropped below the individualized threshold for (2) consecutive sequence repetitions [228]. As NOEXP subjects had no behavior indicative of awareness, the block at which the lowest z score was observed was utilized as the behavioral performance variable. The neural

behavior variable was the block at which the maximal area amplitude occurred for each of the (5) VEP components analyzed. As in our previous experiment [228], the first two blocks of each sequence were not included in the maximal area identification to address neural changes associated with learning the task itself. A Spearman correlation analysis was then conducted for peak area amplitude relative to behavioral performance for each component. Correlation analysis was conducted utilizing R statistics packages *rcorr* and *lmer* [212]. Multiple comparisons for correlations were addressed utilizing Holm corrections and adjusted p values reported.

Statistical Analysis of Frontoparietal Facilitative Network in Priming vs. Transfer Sequence

We have previously demonstrated neural activity suggestive of a facilitative frontroparietal network associated with the development of incidental awareness[228]. This network demonstrates peak neural activity over centroparietal regions (expressed in the P3 amplitude), followed by the left parietal (expressed in the late N1 amplitude), ending with the frontocentral (P2) and frontal regions (early N1). To examine the presence of a facilitative network in both the priming and transfer sequence, a Spearman correlation analysis was conducted for the individual timing of peak area amplitude for all groups. Correlation analysis was conducted utilizing R statistics packages *rcorr* and *lmer* [212]. Multiple comparisons for correlations were addressed utilizing Holm corrections and adjusted p values reported.

Statistical Analysis of Neural Changes Associated with Priming

To assess baseline area amplitude values from Random 1, a one-way ANOVA was conducted for the area amplitude values with between factor of AWARENESS (4 levels: CONTROL, EXP, NOEXP, EXP_NOEXP). Upon verification of no significant difference between groups at baseline, amplitude value changes, relative to R1, were compared between awareness groups in the priming condition. Amplitude values for each region of interest (PCC, LPcun, RPcun, SMA and ACC) were identified for nine points throughout the experiment. Seven of the nine time points were based

on individual neural behavior (blocks at which peak neural activity was noted for the PCC, LPcun, SMA and ACC electrodes), identified as the blocks in which each individual demonstrated peak activity during the priming and transfer sequence. The other two time points were common for all subjects and included the last block of the priming sequence (Block 20) and the random block between the priming and transfer sequences (Random 2). A linear mixed model approach was utilized for each region of interest with factors of awareness (3 Levels: EXP, NOEXP, EXP_NOEXP) and time (9 levels: 7Key PCC, 7Key LPcun, 7Key SMA, 7Key ACC, 7Key End, R2, 10Key PCC, 10Key LPcun, 10Key SMA).

Model 0 (M0) = Repeated measures (RM) with random effect of subject

Model 1 (M1) = RM with fixed effect of time

Model 2 (M2) = RM with fixed effect of group

Model 3 (M3) = RM with fixed effects of time and awareness

An ANOVA compared models to assess the statistical significance for main effects of time and awareness, as well as an interaction effect. Regions of interest demonstrating significant interaction effects were then subjected to subsequent pairwise comparisons for each awareness group to identify the time period of significant difference to Random 1 with single-step Bonferonni corrections for multiple comparisons. Model comparisons utilized the lowest AIC value to identify the best model and adjusted significance levels are reported at $p < 0.05$ for both model and pairwise comparisons.

Visualization of the neural changes occurring between groups over time is displayed utilizing a normalization of area amplitude to address individual differences in VEP amplitudes. Normalization of subsequent block performance was done in a similar manner as Blouin et al. [231], using a ratio formula comparing the area amplitude for each time period of interest to the Random 1 area amplitude. A $\log_2$ transformation was then performed to address the nonlinearity of the ratio results. This provides normalization values such that a positive value reflects an increase in area amplitude relative to the random baseline, while a negative value reflects a decrease in area

amplitude relative to random baseline.

4.4 Results

4.4.1 Behavioral Results: Experiments 1 & 2

Effect of Priming to Explicit Awareness on Generalization to Novel, More Complex Sequence

Nineteen of the thirty-four subjects (55.9%) in the priming group demonstrated behavior predictive of awareness on the 7KEY priming sequence. Ten of these nineteen subjects were subsequently able to accurately repeat the 10KEY transfer sequence, EXP subjects. None of the fifteen CONTROL subjects were able to repeat the 10KEY transfer sequence. These results demonstrate that 52.6% of subjects demonstrating explicit awareness on the 7KEY priming sequence subsequently developed awareness on the 10KEY transfer sequence, while only 6.7% of the NOEXP subjects on the priming sequence and 0% of the CONTROL subjects developed awareness on the 10KEY transfer sequence. A summary of awareness classification breakdown is shown in Figure 4.2. Due to only having two subjects in the NOEXP_EXP awareness group, these subjects were also removed from further analyses, leaving a total of 49 subjects for behavioral analysis. Appearance of behavior reflective of explicit awareness ranged from Blocks 6 to 19 in the priming sequence and from Blocks 4 to 20 in the transfer sequence.

Reliability of the Individualized Threshold in Predicting the Presence of Explicit Awareness

The individualized threshold accurately classified 49 of the 51 subjects from Experiments 1 and 2 as being EXP or NOEXP on the 10KEY transfer sequence. Combining these results with those from Aim 1, provide a 96.1% reliability for indirectly identifying the awareness level of subjects. Subjects inaccurately classified were excluded from further analysis, resulting in 49 subjects for behavioral analysis. The level of reliability observed with 10KEY classification, along with results from Aim 1, was used as supporting evidence for the use of the threshold indicator as a classification method for subjects experiencing the 7KEY priming sequence. The change in latency, relative

to each individual's random baseline performance, for the 7KEY priming sequence is shown in Figure 4.3(a). Note that both the EXP and EXP_NOEXP subjects demonstrate behavior which drops below the threshold of -1.85 z-score.

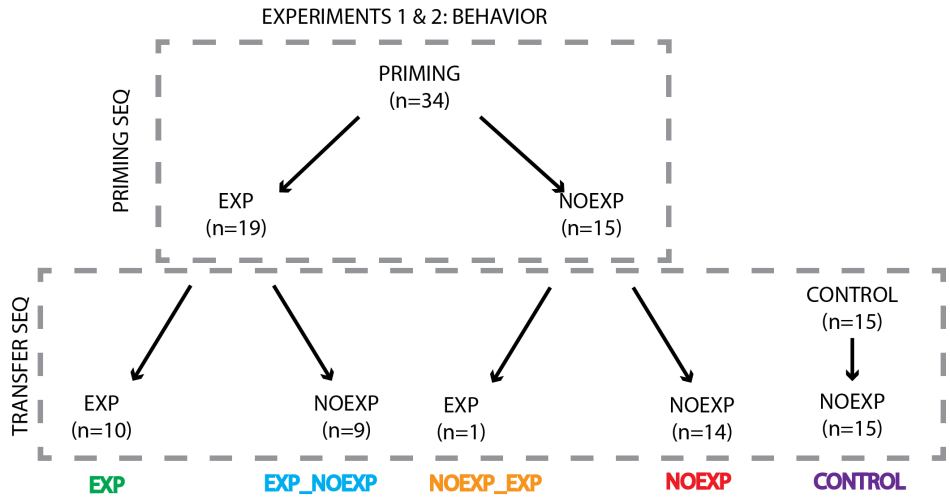


Figure 4.2: **Summary of awareness classification.** Priming and control group awareness classifications.

Effect of Priming on the Level of Recall for the 10KEY Transfer Sequence

Subjects not providing 100% recall on the 10KEY demonstrated a mean percent recall of 30.53% $\pm$ 23.48%. An ANOVA for effect of awareness classification with subjects not demonstrating EXP recall on the 10KEY transfer sequence showed no significant difference in the level of recall ($F(34,2)=0.51$, $p=0.605$). Figure 4.3(b) shows the comparative levels of recall for each awareness group.

Random Block Performance: Latency & Accuracy

The mean latency for random block responses was 398.28 $\pm$ 101.10 ms. Linear mixed model comparisons showed a significant main effect of Random block ($X^2(1 \text{ vs } 2)=33.96$, $p<0.001$) and a significant interaction effect of priming group and random block ($X^2(3 \text{ vs. } 4)=8.57$, $p=0.0138$). Pairwise comparisons showed Random 1 latencies were significantly slower than Random 2 and

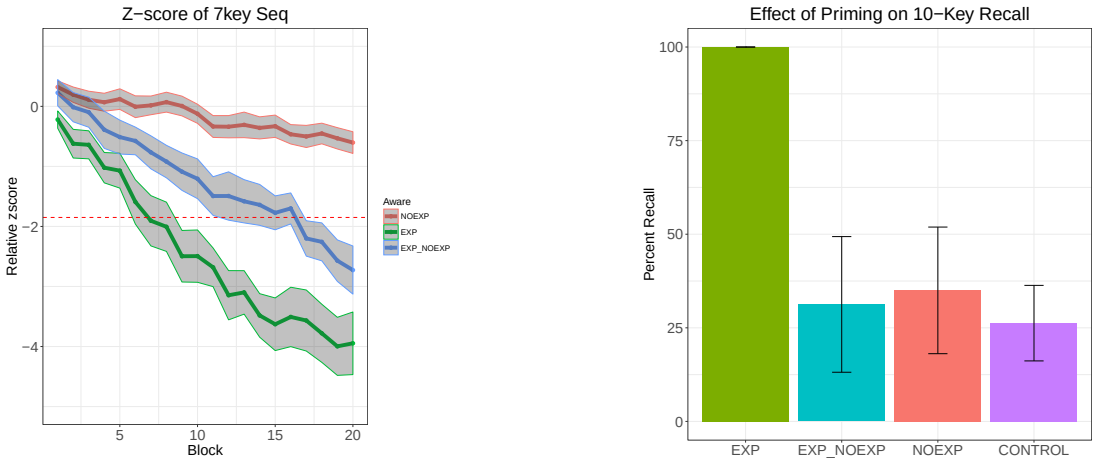


Figure 4.3: **Priming sequence latency change and transfer sequence recall.** (a) Note the change in latency below threshold for both EXP and EXP_NOEXP subjects. (b) Priming without demonstration of EXP awareness on 7KEY does not significantly improve percent recall on 10KEY.

Random 3 for the Priming group. Linear mixed model comparisons for awareness classification showed a significant main effect of Random block (X^2(1 vs 2)=33.96, p<0.001), no significant main effect of awareness, but a significant interaction effect (X^2(1 vs 3)=17.03, p=0.05). Subsequent pairwise comparisons showed that the EXP_NOEXP and the NOEXP groups were significantly faster on Random 2 compared to Random 1.

The mean accuracy for random block responses was 94.92% $\pm$ 21.96%. Linear mixed model comparisons showed a significant main effect of Random block (X^2(1 vs. 2)=18.85, p<0.001), no significant main effect of priming and no interaction effect. Pairwise comparisons showed that Random 1 responses were significantly more accurate than Random 2 and Random 3. Linear mixed model comparisons for awareness classification showed a significant main effect of Random block (X^2(1 vs 2)=18.85, p<0.001), no significant main effect of awareness, but a significant interaction effect (X^2(2 vs 3)=25.29, p=0.001). Subsequent pairwise comparisons showed that the EXP group was significantly more accurate on Random 1 compared to Random 2 and Random 3. Latency and accuracy means for Random blocks by awareness group are shown in Figure 4.4.

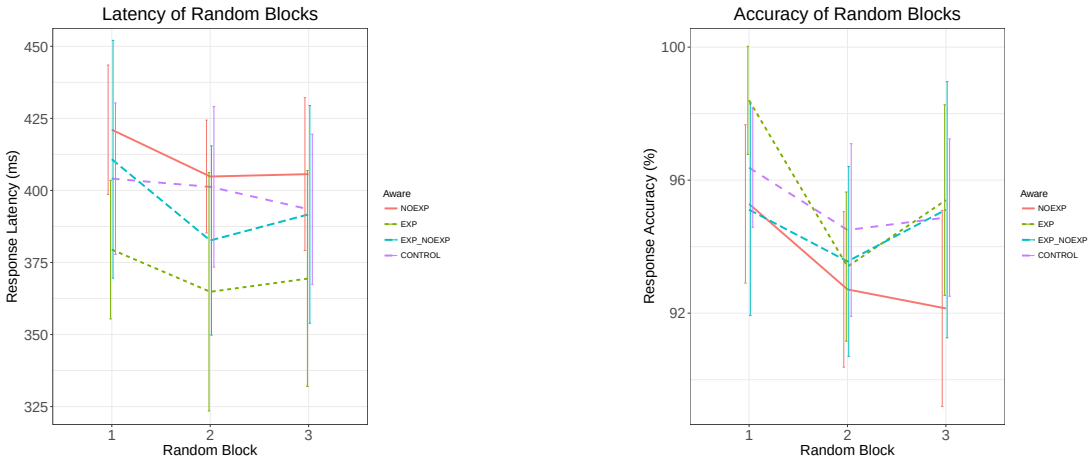


Figure 4.4: **Random latency and accuracy by awareness classification.** Priming group subjects demonstrated significantly faster latencies for Random 2 and 3 compared to Random 1. EXP subjects demonstrated significantly lower accuracy during Random 2.

4.4.2 Neurobehavioral Results: Experiment 2

Twenty-eight subjects participated in Experiment 2 which included an additional cognitive 2-back working memory assessment and EEG data collection during task performance. Nineteen subjects were placed in the PRIMING condition and thirteen of those subjects demonstrated EXP behavior. Of those thirteen subjects, eight were subsequently able to accurately recall the 10KEY transfer sequence. Due to only one subject performing in the NOEXP_EXP group, this subject was removed from subsequent analysis. Awareness classification breakdown is shown in Figure 4.5

Neurobehavioral Results: Working Memory Capacity

Performance on the 2-back working memory (WM) assessment showed a mean adjusted score of 976.05 $\pm$ 336.44 ms. An ANOVA for effect of awareness classification revealed a significant effect of awareness (F(23,3)=3.1883, p=0.04). Pairwise comparisons demonstrated a significant difference in WM performance between the EXP and NOEXP awareness groups, with EXP subjects performing significantly faster than NOEXP subjects. Figure 4.6(a) shows the n-back score summaries.

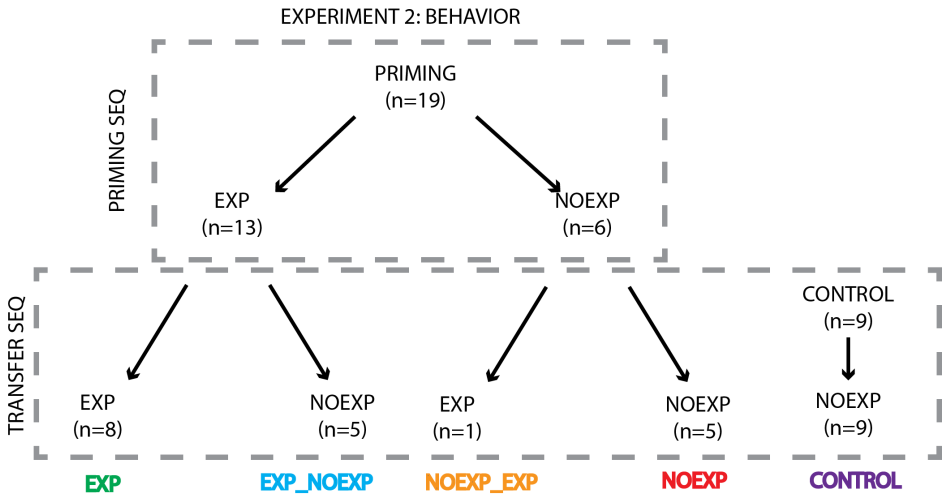


Figure 4.5: **Summary of awareness classification for Experiment 2.**

Neurobehavioral Results: VEP for Random 1 and Random 2

Linear mixed model comparisons for VEP components at Random 1 vs. Random 2 showed differential results depending on the component analyzed. The EarlyN1 showed no significant main effect of awareness, time or interaction effect. The Left LateN1 and P2 components showed a significant main effect of time, with a trend for the Random 2 area amplitude being significantly lower than Random 1. The P3 component showed no significant main effect of awareness or time, but a significant interaction effect with the NOEXP group showing a significant decrease in R2 area amplitude compared to R1. Significance results are summarized in Table 4.1. Figure 4.6(b) shows the interaction effect of the P3 component.

EEG Results: Neurobehavioral Correlations

Table 4.2 and Table 4.3 show the correlations for the timing of VEP component peak area amplitudes with each subject's block of interest (BOI). For subjects not demonstrating EXP behavior on the 7KEY priming sequence or EXP recall on the 10KEY transfer sequence, the BOI was the block at which the subject demonstrated the fastest zscore performance. For subjects demonstrating EXP behavior on both the 7KEY priming sequence and 10KEY transfer sequence, the BOI was identified as the block in which the subject demonstrated response times which dropped below their

Table 4.1: **Significant changes in VEP area amplitude from Random 1 to Random 2**

VEP Component	Main Effect of Awareness	Main Effect of Random Block	Interaction Effect
Early N1 (ACC)	NS	NS	NS
Late N1 (Left Precuneus)	NS	R1>R2, p=0.05	NS
Late N1 (Right Precuneus)	NS	R1>R2, p=0.09	NS
P2 (SMA)	NS	R1>R2, p=0.01	NS
P3 (PCC)	NS	NS	**NOEXP R1>R2, p=0.003**

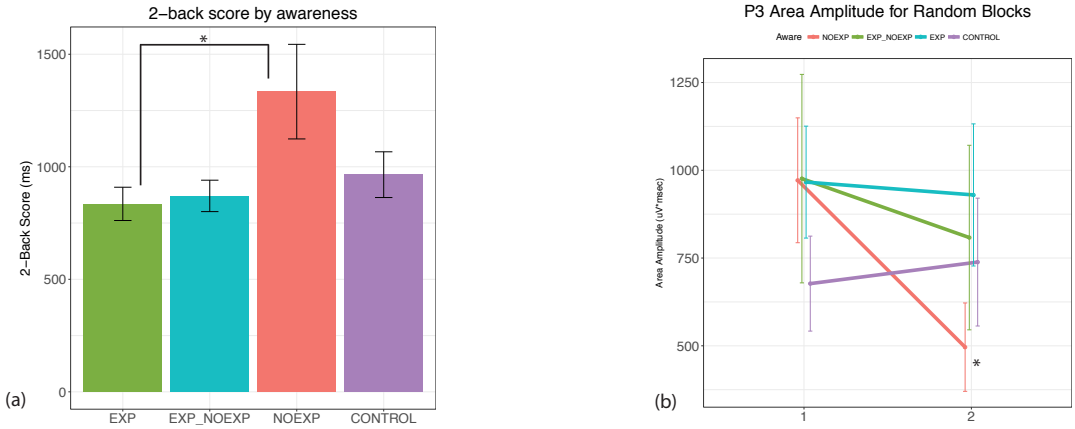


Figure 4.6: **Neurobehavioral measures potentially reflecting working memory capacity.** (a) Summary of 2-back for Experiment 2. Note the elevated 2-back score for the NOEXP subjects compared to the EXP subjects. (b) P3 area amplitude for subjects at Random 2. Note the significantly decreased area amplitude for NOEXP subjects from R1 to R2.

individual threshold for two consecutive sequence repetitions.

Neural Correlations Between VEP Component Peak Area Amplitudes: Facilitative Network Observed with 7KEY Priming Sequence Shifts Upon Transfer

Based on results with the neurobehavioral correlations, the presence of a facilitative network for EXP subjects in the 7KEY priming sequence was examined similar to that seen in Aim 1 [228]. We examined the correlation in timing between the PCC, Left Precuneus, SMA and ACC for peak area amplitude. Figure 4.7(a-b) shows the correlations for both EXP and NOEXP subjects on the

Table 4.2: **Neurobehavioral correlations: 7KEY Priming Sequence (Linear correlation between timing of peak area amplitude and BOI identified by threshold classifier)**

VEP Component	EXP	NOEXP
Early N1 (ACC)	****rho=0.97, p<0.001**	rho=0.28, p=0.59
Late N1 (Left Precuneus)	****rho=0.85, p<0.001**	rho=0.16, p=0.76
Late N1 (Right Precuneus)	***rho=0.57, p=0.04**	rho=0.31, p=0.55
P2 (SMA)	****rho=0.96, p<0.0001**	rho=0.33, p=0.52
P3 (PCC)	****rho=0.57, p=0.04**	rho=0.58, p=0.23

Table 4.3: **Neurobehavioral correlations: 10KEY Transfer Sequence (Linear correlation between timing of peak area amplitude and BOI identified by threshold classifier)**

VEP Component	EXP	EXP_NOEXP	NOEXP	CONTROL
Early N1 (ACC)	rho=0.57, p=0.14	rho=0.7, p=0.19	rho=0.3, p=0.62	rho=0.54, p=0.14
Late N1 (Left Precuneus)	***rho=0.80, p=0.016**	rho=0.05, p=0.93	rho=0.2, p=0.75	rho=0.16, p=0.68
Late N1 (Right Precuneus)	rho=0.59, p=0.13	rho=0.7, p=0.19	rho=0.2, p=0.75	rho=0.65, p=0.057
P2 (SMA)	****rho=0.99, p<0.0001**	rho=0.36, p=0.55	rho=0.8, p=0.10	rho=0.14, p=0.73
P3 (PCC)	rho=0.42, p=0.30	rho=0.70, p=0.19	rho=0.82, p=0.09	rho=0.36, p=0.33

7KEY priming sequence. Figure 4.8(a-d) shows the correlations for all awareness groups with the 10KEY transfer sequence. Table 4.4 and Table 4.5 summarize the linear hypotheses results for both the 7KEY and 10KEY sequences. As was observed in Aim 1, significant linear relationships are seen between all VEP components, with the exception of the SMA and ACC for EXP subjects experiencing the priming sequence. Significant correlation is lost between the LPcun and the PCC for EXP subjects in the transfer sequence.

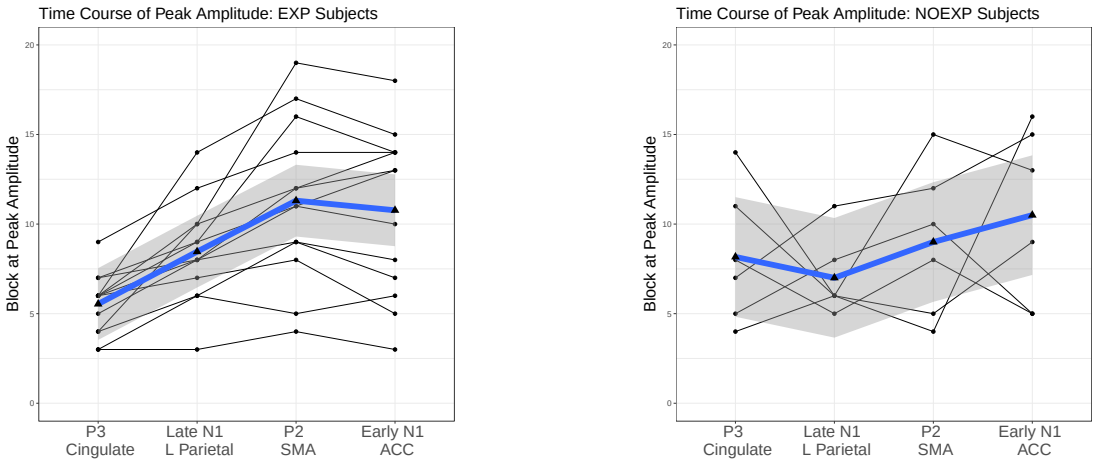


Figure 4.7: **Neural correlations between VEP component peak area amplitude for 7KEY priming sequence.** (a) EXP subjects demonstrate a significant linear relationship between all components except SMA & ACC. (b) NOEXP subjects do not demonstrate significant linear relationships between components.

Table 4.4: **Correlation matrix for EXP and NOEXP groups in the 7KEY priming sequence.**

Linear Hypotheses	EXP	NOEXP
$LateN1 - P3 = 0$	***p=0.02**	p=0.86
$P2 - P3 = 0$	****p<0.001**	p=0.88
$EarlyN1 - P3 = 0$	****p<0.001**	p=0.28
$P2 - LateN1 = 0$	****p<0.001**	p=0.99
$EarlyN1 - LateN1 = 0$	**#p=0.0664**	p=0.77
$EarlyN1 - P2 = 0$	p=0.44	p=0.61

Table 4.5: **Correlation matrix for all awareness groups in the 10KEY transfer sequence.**

Linear Hypotheses	EXP	EXP_NOEXP	NOEXP	CONTROL
$LateN1 - P3 = 0$	p=0.99	p=0.56	p=0.74	p=0.83
$P2 - P3 = 0$	**#p=0.08**	p=0.17	p=0.94	***p=0.02**
$EarlyN1 - P3 = 0$	***p=0.02**	p=0.41	p=0.98	p=0.80
$P2 - LateN1 = 0$	***p=0.03**	p=0.88	p=0.97	p=0.20
$EarlyN1 - LateN1 = 0$	****p=0.006**	p=0.99	p=0.91	p=0.99
$EarlyN1 - P2 = 0$	p=0.93	p=0.99	p=0.22	p=0.22

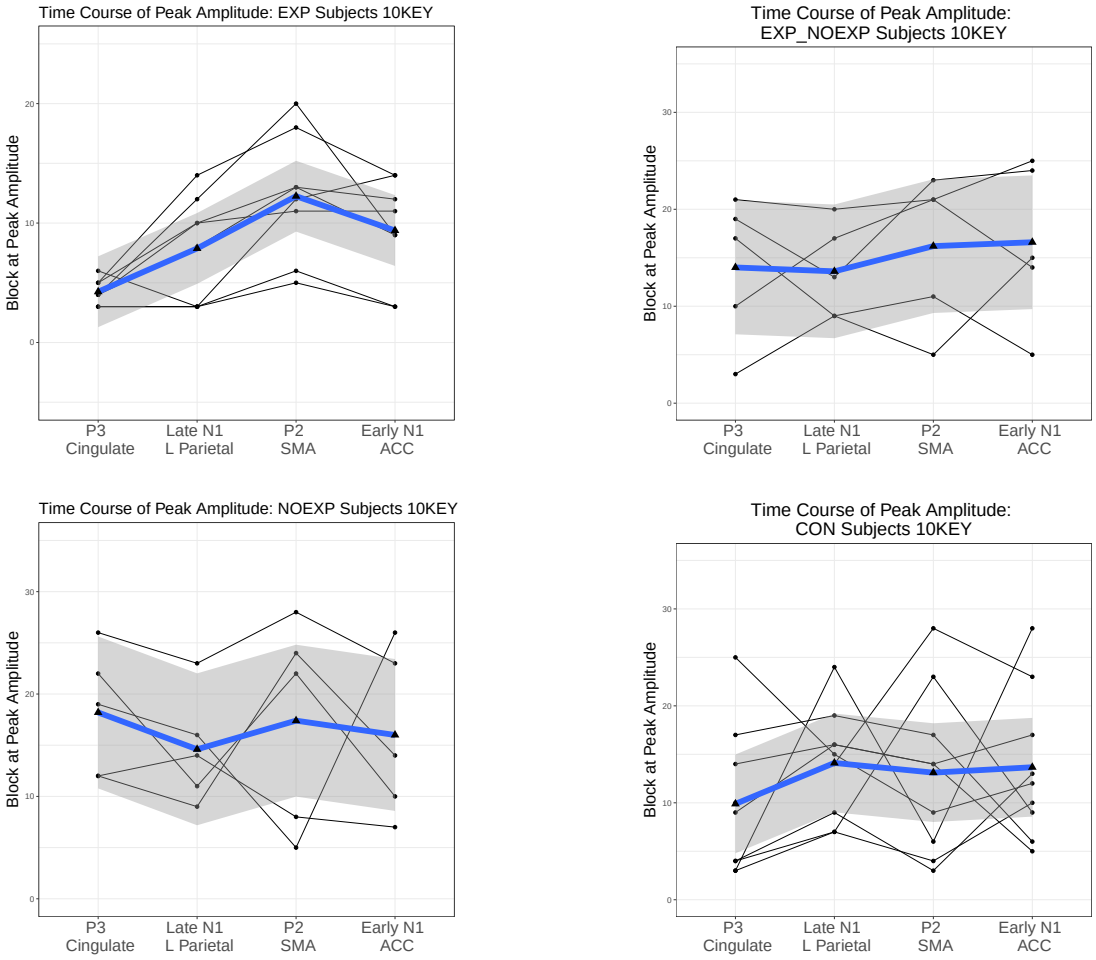


Figure 4.8: **Neural correlations between VEP component peak area amplitude for 10KEY transfer.** (a) EXP subjects linear relationships between PCC & SMA, along with PCC & Left Precuneus are no longer present. (b-d) EXP_NOEXP, NOEXP and CONTROL subjects do not demonstrate significant linear relationships between components.

Table 4.6: **Neural changes for ROI's over time between groups.**

Model Test	Analysis	df	PCC	LPcun	RPcun	SMA	ACC
$Random1$	Effect of Awareness	2	p=0.93	p=0.40	p=0.56	p=0.54	p=0.59
$M0$	Repeated measures	3					
$M0vs.M1$	Main Effect of Time	12	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
$M0vs.M2$	Main Effect of Awareness	5	p=0.45	p=0.27	p=0.24	p=0.10	p=0.96
$M2vs.M3$	Interaction Effect	32	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**

Neural Changes Over the Course of Priming and Transfer Sequences

Initial analysis of Random 1 neural activity revealed no significant difference in area amplitude between awareness groups at Random 1 (Table 4.6 and Figure 4.9(a)). Linear mixed model comparisons for changes in neural activity for regions of interest (PCC, LPcun, RPcun, SMA, and ACC) over time are shown in Table 4.6 and Figure 4.9(b-f). All regions of interest showed a significant main effect of time, no significant main effect of awareness and an interaction effect of awareness over time.

For the PCC (Figure 4.9(b)), EXP subjects demonstrate a significant decrease in PCC amplitude relative to Random 1 over all time periods with the exception of the block of peak SMA activity and Random 2. All three groups demonstrate a significant decrease in PCC activity by the end of the 7KEY priming sequence compared to Random 1.

For the SMA (Figure 4.9(c)), both the EXP and EXP_NOEXP groups demonstrate significant amplitude, relative to R1, at the block of peak SMA activity. The NOEXP group did not demonstrate significant differences in SMA amplitude, relative to Random 1, at any time period over the course of the experiment.

For the LPcun and RPcun (Figure 4.9(d) and (e)), only the EXP_NOEXP group demonstrated a significant increase in activity relative to Random 1. This significant increase occurred at the block

of peak LPcun activity during the 7Key priming sequence.

For the ACC (Figure 4.9(f)), the EXP group demonstrated significant increases in amplitude, relative to Random 1, at three time periods: (1) the block of 7Key peak LPcun activity, (2) the block of peak ACC activity, and (3) the block of peak 10Key LPcun activity. The EXP_NOEXP group demonstrated significant increase in ACC amplitude, relative to Random 1, only at the block of peak ACC activity. The NOEXP group did not show any significant difference for ACC amplitude, relative to Random 1, at any time period over the course of the experiment.

4.5 Discussion

The overarching goal of Aim 2 was to examine the effect of incidentally developed explicit awareness on generalization to a novel, more complex sequence. Using the individualized indicator from Aim 1 for the presence and timing of explicit awareness developed incidentally, subjects could be classified as learners or non-learners of either the priming 7KEY, or more complex, transfer 10KEY sequence. For the priming, 7KEY sequence, over 50% of the subjects demonstrated explicit behavior, while for the 10KEY sequence, none of the CONTROL subjects demonstrated explicit awareness, demonstrating the impact of sequence length on awareness development seen in Aim 1 [228]. Of the priming subjects displaying explicit behavior on the 7KEY sequence, 50% demonstrated explicit recall on the 10KEY, supporting results of previous studies demonstrating the beneficial effects of priming on motor learning [232, 233]. A practice effect of priming was also noted, demonstrated by the significantly faster reaction times shown by the priming group on the second random block. Priming did not, however, significantly improve the level of recall for subjects failing to develop full explicit awareness, suggesting that if, or how, the priming sequence was learned is an important factor in the effects of priming on generalization. The fact that not all subjects in the priming group demonstrated learning of the 10KEY transfer sequence placed subjects into four major groups: (1) learners of the priming sequence who could generalize and learn the 10KEY sequence (EXP), (2) learners of the priming sequence who were unable to successfully transfer to the 10KEY sequence (EXP_NOEXP), (3) subjects who did not demonstrate learning of

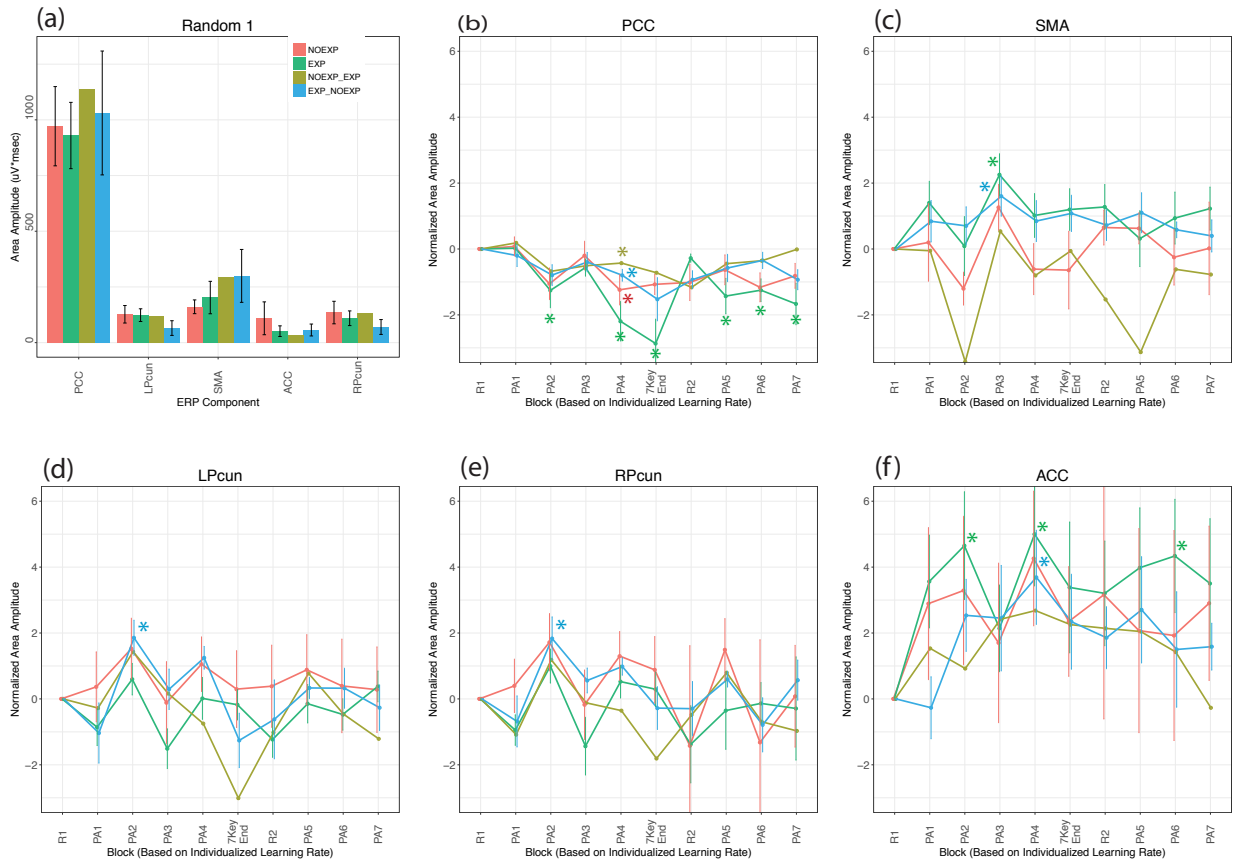


Figure 4.9: **Neural changes over time, relative to Random 1 baseline, for each region of interest.** (a) Initial area amplitudes at Random 1 for each region of interest shows no significant difference between Awareness groups. (b-f) Shows normalized area amplitude for each region of interest over the course of the experiment. Time periods indicate blocks at which each subject demonstrated peak activity reflective of individual learning rates. All indicated significant differences (p¡0.05) are relative to Random 1 area amplitudes.

the 7KEY or the 10KEY sequence (NOEXP), and (4) subjects not experiencing the 7KEY priming sequence (CONTROL). Neurobehavioral differences between these groups suggest that learning strategy and working memory capacity may play a role in a person's ability to learn a new motor sequence to the level of explicit awareness, and subsequent ability to transfer learning to a more complex sequence. The differences noted may contribute to some of the variability seen with individuals experiencing a rehabilitative intervention. These results provide potential targets for interventions to improve the efficacy of treatments in motor learning.

4.5.1 Assumptions and Limitations

All priming subjects demonstrated a decrease in latency over the course of the priming sequence, which was significantly increased upon introduction of the second random sequence. Prior sequential motor learning studies have utilized this method to demonstrate that the increased speed was related to the presence of a repetitive pattern as opposed to general visuospatial mapping [58]. This behavioral response to the random block at the end of the 7KEY supports the assumption that implicit learning was present for all priming subjects, regardless of their awareness classification. The timing, and presence, of explicit awareness, for both the priming and transfer sequence, was identified through the use of an individualized indicator identified in a previous study by our lab [228]. As the actual level of recall was never assessed for the priming sequence, high reliability of the indicator in accurately classifying subjects is necessary to support awareness level assumptions made by the indicator's classification. When combined with the results from Aim 1, the individualized threshold indicator presents with sensitivity and specificity measures of 90.9% and 90% respectively for 72 subjects (See Figure 4.10). The additional assessment of asking subjects to recall the pattern one block after demonstrating explicit behavior, provides evidence that the indicator is effective in identifying both if, and when, a subject has developed awareness.

Based on the facilitative network identified in Aim 1, Aim 2 had an a priori focus of attention on five specific neural regions of interest for VEP analysis (PCC, LPcun, RPcun, SMA, and ACC) which demonstrated significant neurobehavioral correlation with behavior predictive of explicit

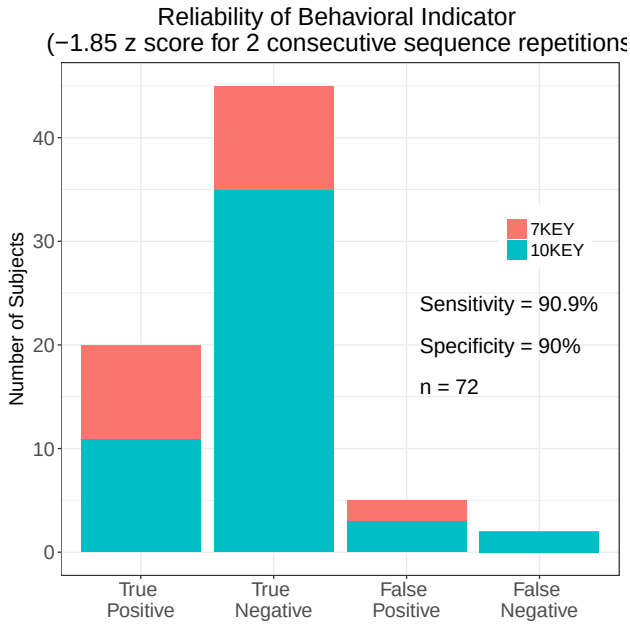


Figure 4.10: **Reliability of the individualized threshold from Aim 1 and Aim 2.** The individualized threshold produced a 90.9% and 90% sensitivity and specificity respectively, for accurately classifying 72 subjects as EXP or NOEXP.

awareness. While these regions are noted to be involved in visuomotor learning, other regions have been also been shown to play a role in motor learning and predictive motor coding. Multiple studies have shown involvement of deeper structures such as the insula, basal ganglia, and cerebellum which are beyond the scope of EEG analysis [234, 235, 40, 45].

Finally, Aim 2 utilized a fairly simple finger-tapping task. Most daily activities involving sequential movements are much more complex, involving multiple joints. Therefore, Aim 3 explored the utilization of the proposed facilitative visuomotor integration network during the acquisition of more complex skills. This approach allowed for assessment of additional behavioral attributes, of eyetracking and kinematics, to further elucidate the visuomotor integration during awareness development.

4.5.2 Development of awareness with the priming sequence: Presence of facilitative frontoparietal visuomotor integration network

Thirteen of the nineteen priming subjects in Experiment 2 demonstrated explicit behavior during exposure to the priming sequence. Utilizing the peak amplitude VEP analysis method as described in Aim 1 provided similar results for these EXP subjects [228]. EXP subjects demonstrated significant neurobehavioral correlations of the timing for the examined VEP component peak activity with the timing of behavior identified by the individualized indicator as the existence of explicit awareness. Additionally, neural correlations of the timing for peak amplitude between these neural regions demonstrate a similar sequential, linear relationship supporting the hypothesis from Aim 1 of a facilitative frontoparietal visuomotor integration network involved in the development of explicit awareness [228]. These linear relationships provide additional support for a progression of peak activation from the PCC to the left and right PCun, with the left SMA and ACC peak activity appearing together as shown in Figure 4.7(a). These neurobehavioral and neural correlations were not present for the NOEXP subjects, providing evidence that the activation of this visuomotor network is required for awareness to occur in the priming sequence.

4.5.3 Enhanced SMA and ACC activity for EXP and EXP_NOEXP subjects reflect presence of awareness

Neural activation patterns between groups demonstrate that both EXP and EXP_NOEXP subjects show significantly greater SMA activation, compared to Random 1, at the block of individual peak SMA activity. The SMA activity for NOEXP subjects did not vary significantly from that of Random 1. This supports that the peak SMA amplitude is reflective of an internally-generated execution of the motor sequence. Additionally, EXP and EXP_NOEXP subjects showed significantly greater ACC activation, relative to Random 1, at the block of individual peak ACC activity, while NOEXP subjects failed to show a significant increase in ACC activity. This supports studies that have shown ACC involvement during the performance of controlled actions as would be utilized

in an explicit execution of the learned sequence [236, 237, 238, 139, 138].

4.5.4 Recognizing a pattern: The role of the PCC and working memory

VEP amplitude changes over time revealed striking differences between the three awareness groups for all five neural ROI's. Both the timing and location of these differences suggest the presence of strategy differences between the groups which either facilitated or inhibited the ability to acquire the motor sequence to a level of explicit awareness for the priming sequence. The proposed facilitative network begins with the PCC which is noted to involved in the identification of regularities in the environment which warrant a behavioral strategy shift for more effective accomplishment of the current task [113]. The identification of a regularity involving a repetitive sequence relies on functioning predictive learning processes and visuospatial working memory capacity [239, 230]. The PCC has anatomical connections with the medial temporal lobe, precuneus and basal ganglia, all known to be involved in statistical learning, making it well positioned to facilitate such identification of regularities [113]. The strategy shift provided by this pattern recognition may involve recruiting a learning strategy to identify the statistical nature of the regularity [140, 111, 240]. This shift may be seen at the block of peak LPcun activity when EXP subjects demonstrate both a significant decrease in PCC activity and a significant increase in ACC activity, while EXP_NOEXP subjects demonstrate a significant increase in bilateral precuneus activity. The lack of a significant change in any of the aforementioned regions by the NOEXP subjects suggests that these subjects did not change their approach to the task and remained in a stimulus-response mode throughout the experiment.

The maintained stimulus-response behavior by NOEXP subjects may be explained by working memory capacity. As stated earlier, the PCCs ability to identify relevant environment regularities relies on associative learning and spatial attention, both of which require an effective visuospatial working memory capacity [241, 112, 113, 111]. To successfully execute the priming sequence, subjects must be able to identify and concatenate each of the 7-elements. This requires the ability to encode, maintain and retrieve the individual elements through utilization of visuospatial working

memory processes [152, 239, 154, 156]. A reduced working memory capacity could prevent the PCC from identifying the presence of a statistical regularity relevant enough to warrant a shift to a learning strategy. Performance on the 2-back task, along with the reduced P3 amplitude during the second random block, suggests NOEXP subjects entered the experiment, and maintained, a cognitive state which impacted their functional working memory capacity [242, 243, 201]. Multiple factors have been shown to impact a persons functional working memory capacity including mood, stress, sleep deprivation, secondary cognitive demands, recent exercise, attention-enhancing drugs and resting state connectivity [244, 245, 246, 247, 248, 249, 250, 251]. Taken together, these results reinforce previous research which has correlated functional working memory capacity to subsequent motor learning performance [2, 230, 252, 158], suggesting that the functional working memory capacity a person has at the start of a rehabilitative session must be taken into consideration when determining the appropriate difficulty level for that session. Further studies examining the effect of reducing sequence length for subjects demonstrating a reduced working memory capacity would provide additional insight into the role working memory has on shifting to a learning strategy.

4.5.5 The role of learning strategy in facilitating transfer: Importance of recruiting the cingulate

Previous studies of motor learning have proposed two strategies for learning based on probabilities: (1) matching the exact statistics related to the sequence (memorizing the sequence), and (2) maximizing the most probable outcome (identifying current probabilities based on priors) [253, 254, 255]. Giorgio et al. recently provided evidence that unique brain networks are involved for the two strategies [256]. Subjects utilizing a matching strategy demonstrated higher bilateral precuneus activation, while subjects utilizing a maximizing strategy demonstrated elevated striatal and frontal activation. EXP_NOEXP subjects show significantly elevated amplitudes for both the LPcun and RPcun at the block of peak LPcun activity, suggesting a reliance on the aforementioned memorizing strategy for learning the sequence. EXP subjects, alternatively, show significantly higher ACC activation at the block of peak LPcun activity. This would suggest the use of the maximizing

strategy for EXP subjects. The importance of the strategy selection becomes most apparent upon transfer to the new, more complex sequence. The use of a maximization strategy by EXP subjects facilitates discovery of the 10-KEY sequence, while the use of a matching/memorization based strategy by the EXP_NOEXP subjects fails when exposed to the longer, 10-KEY sequence upon transfer. This failure may be, as with the NOEXP subjects, attributed to working memory and interference. It is well established that performance on SRT tasks is related to sequence lengths [83, 73, 149, 54, 59, 150, 147]. As the transfer sequence is longer than the 7-element maximum seen from our previous work [228], it is not surprising that EXP_NOEXP subjects were unable to learn the full sequence pattern through utilization of a memorization strategy method.

EXP subjects, however, are proposed to benefit from either starting with, or shifting to the maximization strategy, which is suggested to involve a cingulo-striatal network [256]. The ACC has long been implicated in a variety of cognitive tasks including attentional gating, decision-making and cognitive control motor strategies [140, 240, 257]. Specifically, ACC activity is known to increase when the current behavior is identified as suboptimal and an alternative behavior should be considered. As the ACC has connections to both the precuneus and striatal regions, it is well placed to play a critical role in identifying the suboptimal method of memorizing and shift to a more associative learning strategy. The subsequent recruitment of the striatum may allow for an integration of both endogenous and exogenous sources of information relevant for the sequence learning. Within the context of the current experiment, this integration may present as a balance between the visual information provided by the external cue and sensorimotor information provided by the motor output of the button press for identification of the sequence. In addition to the associative learning benefit provided by recruitment of the striatum, involvement of the basal ganglia has been noted to be pivotal for consolidation of recently learned motor tasks, reducing the impact of interference of a new task [45, 40, 36]. Putative involvement of a cingulo-striatal network would explain the ability of EXP subjects to successfully acquire explicit awareness of the priming sequence, while also being able to acquire awareness of the rapidly introduced, more challenging transfer sequence. As the current study did not examine subcortical structures specifi-

cally, additional studies utilizing the spatial resolution of fMRI may help elucidate the role striatal structures play in this type of incidental discovery-driven motor learning.

A putative cingulo-striatal involvement may also contribute to the EXP_NOEXP subjects performance on the transfer task as they experience the detrimental effects of interference due to the fragility of the recently memorized 7-KEY sequence. Studies examining recruitment of an explicit strategy during motor learning may encourage the use of a memorization strategy, interfering with the cingulo-striatal system impacting memory consolidation [258, 226]. The utilization of a memorization strategy may explain results from previous studies which have shown that explicit awareness developed through an intentional strategy is detrimental to generalization [54, 227, 80].

4.6 Conclusions

The results of Aim 2 provide insight into potential factors contributing to individual variations observed in motor learning. The importance of examining performance from an individual perspective was, as in Aim 1, highlighted by the wide range in learning rates for the EXP and EXP_NOEXP subjects in both the priming and transfer sequences. The reliability of the individualized indicator was reaffirmed with the results of the transfer sequence, producing an overall reliability of over 90% accuracy in classifying subjects as explicitly aware. Asking for recall immediately after explicit behavior was identified in the transfer sequence provided evidence that the threshold was effective in identifying both if, and when, a subject had developed awareness. Utilization of the indicator provided an opportunity to identify neural changes correlative with each individual's learning rate. This method revealed neural activation differences which provide evidence that working memory capacity and learning strategy, have an influence on neural systems which affect learning quality and generalizability.

During the priming sequence, subjects displaying explicit behavior demonstrated neurobehavioral evidence of the same learning-dependent facilitative fronto-parietal network observed in Aim 1. The addition of a transfer sequence produced two groups of subjects, EXP and EXP_NOEXP, allowed an opportunity to examine how this facilitative network was involved in successful gener-

alization of a motor skill. The use of the individualized indicator of learning from Aim 1 provided neurobehavioral correlation evidence for limiting factors to both initial learning of a sequential motor task and the ability to generalize to a novel, more complex motor task. Initial learning may be prone to working memory capacity issues, which cannot be easily overcome. Generalization abilities appeared to be affected by the learning strategy adopted impacting which network was recruited during learning. A memorization, precuneus-driven strategy inhibited generalization, while a maximization, striatally-driven strategy facilitated generalization.

The variability in learning rates, along with the facilitation of neural activation patterns revealed in Aim 2 highlight the importance of examining performance from an individual perspective both in research and in practice. Recent studies have provided evidence for efficacy of tDCS [259, 260, 261, 262], caffeine or other attentional-enhancing medications [263, 245, 248] and short aerobic bouts prior to testing [250, 264], in improving attention and working memory capacity. Further studies may reveal whether individuals in a reduced working memory capacity state, may benefit from these interventions or from exposure to a shorter, less complex motor sequence resulting in the consolidative benefits seen with our EXP group. From a strategy perspective, individuals demonstrating recruitment of a memorization, precuneus-driven strategy for learning a motor sequence, may benefit from a consolidation period prior to introducing a novel, or more complex sequence to reduce interference effects. Additional studies aimed at examining the potential efficacy of these neural markers as a tool to individualize practice patterns could provide valuable information for individuals working in development, athletic and rehabilitative fields.

The paradigm utilized in both Aim 1 and Aim 2 utilized a fairly simple finger-tapping task. As most daily activities involve more complex, reaching movements, Aim 3 explored sequential learning utilizing a three-dimensional reach-and-grasp task. Extending the sequential task into this space, allowed for a more precise measure of kinematic and gaze behaviors during the experiment. This approach introduced an opportunity to examine changes within the facilitative visuomotor integration network occurring during sequential learning associated with both visual and motor behavioral changes.

CHAPTER 5
SPECIFIC AIM 3

5.1 Introduction

The purpose of Aim 3 was to examine sequential motor learning in a three-dimensional workspace to allow for a more precise examination of the kinematic and visual contributions during sequential learning. Results from Aim 1 and 2 provided evidence for a facilitative network during the incidental development of explicit awareness. The proposed facilitative network revealed in both Aims 1 and 2 involved the LPcun after peak PCC activity, as well as significant behavioral correlations with RPcun peak activity. As these two areas are attributed to visuomotor planning and visuospatial awareness, the correlations found provide evidence that visual information played a pivotal role in the development of awareness. The additional results for generalization from Aim 2 introduced the potential role of a cingulo-striatal network for subjects experiencing successful generalization. These results suggest a shift from a visual, precuenus-driven method for learning the sequence, to one which utilizes striatal structures to incorporate both visual and sensorimotor information for associative learning of the sequence. To decouple visual and sensorimotor contributions during sequential learning separate measures of gaze and kinematic behavior were accomplished through the utilization of eye-tracking and a three-dimensional reach-and-grasp sequential learning task. The inclusion of a reach-and-grasp task also introduces an activity that is more similar to the complex kinematic tasks utilized in daily living. This provides the opportunity to explore potential barriers for patient populations who demonstrate reduced sequential learning. Our lab has previously demonstrated that sequential motor learning is hindered when using a prosthetic device [12], potentially due to the enhanced visuospatial demands of controlling the device, therefore, Aim 3 sought to understand the barriers through comparing performance variables for intact subjects both with and without a prosthetic device, and upper limb amputees.

Eye-tracking is a well established method for identifying where the visual focus of attention is localized during accomplishment of a task. Two key parameters often examined with eye-tracking include saccade and fixation behavior. Saccades are rapid point-to-point eye movements which provide the opportunity to change where the area of foveal attention is located. There is no visual information provided to the visual system during a saccadic movement. Visual fixations are utilized to keep the eye steady while gathering visual information, and typically last 100 to 150 ms. Recent studies have demonstrated that saccade behavior is flexible depending on the task demands and prior exposure, with changes noted during motor learning [78, 265, 30, 76, 266]. It is suggested that the changes in saccade behavior, both latency and the number of saccades, are necessary for successful identification of target features and utilized in online identification errors in motor execution. In addition to saccade behavior, the latency of visual fixation has also been utilized as a measure to indicate the presence of reactive versus predictive gaze behavior. Multiple studies have demonstrated the presence of anticipatory visual fixations correlated with the development of sequence awareness [267, 268]. The proposed advantage of such predictive gaze behavior is to allow for additional time for motor planning prior to movement execution [269, 268, 270].

While the utilization of reaction time as measure of motor learning is valuable, it combines two important, yet separate, aspects of motor execution. A measure of reaction time, by its nature, combines the time required to initiate a movement (movement onset) and the subsequent time required to accomplish the movement execution (movement time). The typical finger-press SRT task paradigm does not allow for the separation of movement onset and movement time, as they occur almost simultaneously. The introduction of a reach aspect to the task provides the ability to monitor when movement begins, along with the time required to reach the desired target. A study by Ghilardi et al. demonstrated that during implicit learning of a reaching sequential motor task, movement onset times decreased, while movement time remained relatively stable [23]. It is proposed that this anticipatory movement onset behavior provides the opportunity to spend more time on the actual motor execution. According to the well-known speed-accuracy-tradeoff [271], this increased time for movement should increase the accuracy of movement execution.

While the study by Ghilardi demonstrated this tradeoff during implicit learning, subjects did not develop explicit awareness. A subsequent study examining the effect of explicit awareness on movement time, demonstrated enhanced movement vigor associated with awareness [56]. Both of the studies above, however, examined the behavioral attribute of time only and constrained movement to a two-dimensional workspace, impacting the ability to examine the potential cost of speed on the kinematic smoothness during movement execution. The incorporation of kinematic measures reflective of movement quality, during a three-dimensional task, provides additional information regarding behavioral changes associated with incidentally developed awareness during motor learning.

The paradigm of Aim 3 did not limit the task to a reaching movement only, but rather included a grasp and transfer aspects as well. While subjects had to transfer the disc to a new location, analysis of Aim 3 is limited to the reach-and-grasp portion of each trial. The requirement of a grasping component, introduces an important level of complexity for the task. The ability to pick up an item requires coordination of a reaching movement along with the act of grasping the desired object. The process of reaching first requires a visuospatial transformation regarding the externally-provided visual information about the target location with respect to the current location of the limb which will accomplish the reach [272, 273, 274]. This information must then be transformed into a motor command to activate the appropriate muscles for achieving the end point target. The grasp component, on the other hand, requires additional information regarding the size, shape, texture, density of the object to be picked up. This information is utilized to establish a motor command which will apply the appropriate grip orientation, aperture, and force upon grasping the object. The motor plan for both the reach-and-grasp process will also include any expected sensorimotor outcomes if the task is successfully completed. The visual expectation will be focused on seeing the hand, or device, at the target location within the timeframe expected for reach trajectory [273]. The sensorimotor outcome will primarily involve information regarding the tactile feedback accompanying the fingers making contact with the object [275]. When using a prosthetic, this tactile sensory information is not available, increasing the reliance on other sensory

information to identify when the grasp phase has been completed [12, 159, 276]. Having experience with such sensory outcomes provides the ability to develop expected sensory outcomes for future motor actions. As proposed by the internal model, expected sensory outcomes for a given motor plan can be utilized as a comparator for incoming sensory information for the identification, and correction of errors online [125]. This error information can then be utilized to update the motor plan for the next time. It is proposed that this error-based learning is a common mechanism for the development and improvement during motor skill acquisition [277, 278].

The final inclusion of EEG measurements allowed for neurobehavioral correlations to identify how visual and sensorimotor information contributes to, or inhibits, the ability to learn a motor sequence to the level of explicit awareness. Areas of interest include the neural regions identified in Aims 1 and 2, the PCC, LPcun, RPcun, SMA and ACC to examine if the same facilitative network is utilized with discovery of a three-dimensional sequential task. An additional area of interest, the right prefrontal gyrus was added to explore the role or error-based learning in awareness development. Multiple studies of error correction and error-based learning have shown the pivotal role of the right prefrontal gyrus in the processing of sensory-based errors during movement execution [279, 278, 280, 277].

5.2 Hypotheses

The multimodal approach of Aim 3 resulted in multiple hypotheses regarding behavior, kinematics, gaze and neural activation. Overall, it was hypothesized that prosthesis users would demonstrate reduced sequential motor learning compared to controls, along with neurobehavioral measures indicative of enhanced visuospatial demands.

Behaviorally, it was hypothesized that the behavior measure of movement onset would be a reliable indicator for the presence of incidentally developed awareness. Studies of visual processing have indicated a processing delay of over 100 ms for reaction-based movement, therefore, movement onsets faster than that could be assumed to be predictive or impulsive. Inclusion of the additional requirement of movement onset times with an upper confidence level faster than 110 ms

for the consecutive sequence repetitions, as with Aims 1 and 2, was hypothesized to be reflective of behavior indicated of full sequence awareness.

Gaze behavior changes expected to be reflective of oculomotor sequence learning included reduced saccade latency and reduced gaze onset latency. It was expected that gaze onset latencies that were negative relative to stimulus onset time for all target locations would be predictive of explicit sequence awareness. As saccade behavior is reflective of changes in visual fixations, it was hypothesized that subjects utilizing a prosthetic device would demonstrate a greater number of saccades during the reach to compensate for the reduced sensorimotor information available at the terminal device during grasp.

Kinematic changes expected to be reflective of sequence learning included changes reflective of shifts in the speed-accuracy-tradeoff, with accuracy measures focused on movement quality. Movement smoothness was measured as variation in velocity during the reach, which was hypothesized to improve with sequence learning as more time was provided for movement execution due to the presence of anticipatory movement onset. It was expected that changes in the initial reach direction, compared to the current target goal, would indicate an error in reach direction and be reflective of sequence learning. Finally, it was hypothesized that subjects using a prosthesis would participate in enhanced trunk movements to compensate for the reduced pronation and supination imposed by the prosthetic device or amputation.

From a neural perspective, it was hypothesized that prosthesis users would demonstrate differential neural activity over left and right precuneus regions, reflective of enhanced visual reliance due to the reduced proprioceptive/tactile information available compared to healthy, intact subjects.

5.3 Equipment

5.3.1 Fictive Amputee Model System

Specific Aim 3 uses a fictive amputee model system (FAMS) created in our lab to allow healthy, intact subjects to complete the task while utilizing a prosthetic device. Prior studies in our lab have demonstrated similar neural and kinematic behaviors between amputees and intact subjects

when performing a task with their respective prosthetic devices [175, 281, 12]. The FAMS used in this dissertation was a voluntary-open device with minimal ferrous magnetic components to ensure compatibility with the electromagnetic motion detection system utilized. The device was fitted for each subject using Velcro pads to tighten the device on the right forearm, minimizing pronation and supination capabilities. Additionally, the cable harness strap was adjusted for each subject to allow the FAMS device to open when the arm was 150 degrees of full extension. Opening the device before that level of extension, therefore, required the use of the contralateral, left shoulder pulling the cables to open the lateral jaw. The medial jaw remained fixed in position throughout use of the device. Figure 5.1 shows the FAMS device used.

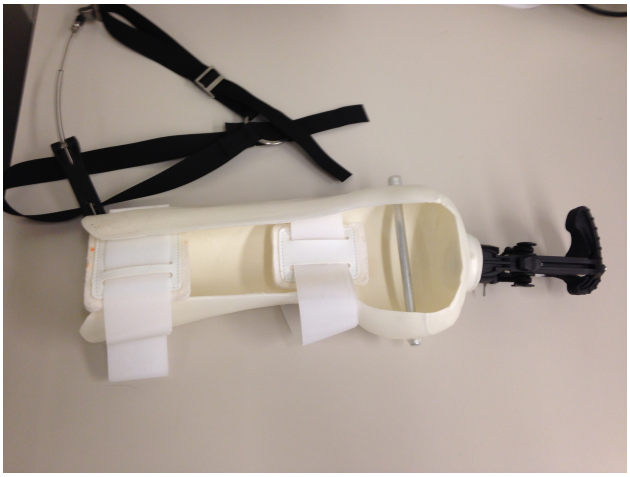


Figure 5.1: **Fictive Amputee Model System (FAMS).** Fictive amputee model system (FAMS) used for specific Aim 3. The device was used by healthy, intact subjects to examine sequential learning while using a prosthetic device.

5.3.2 Eye-tracking

For specific Aim 3, eye-tracking was collected to provide behavioral data regarding the perceptual component of sequential learning. Eye-tracking is a recent analysis tool developed based on well-known characteristics of eye movements and interpretation relies on the eye-mind assumption proposed by Just et al. [282]. This assumption proposes that eye movements provide a dynamic trace of where attention is being directed. Eye movement characteristics utilized in eye-tracking analysis rely on the how eye movements serve to assist in visual perception.

Visual perception involves three major regions of the eye: (1) foveal, (2) parafoveal, and (3) peripheral [283]. The foveal region is the central area of the retinal field which provides the greatest acuity of visual information. The parafoveal region surrounds the fovea and has decreased acuity relative to the foveal region. The peripheral area is outside the parafoveal region and provides visual information with poor acuity. We tend to move our eyes to locate objects in the foveal region [284]. Eye movements are classified as saccades, smooth pursuit, and fixations. Smooth pursuit attempts to keep the fovea on a moving target, while fixations attempt to stabilize the eye during head movements. Saccade eye movements are classified as quick eye movements between fixations. No new visual information is acquired during saccades [285].

The eye tracker utilized in this dissertation, designed by Pupil Labs (Pupil Labs UG, Berlin, Germany), combines camera images from three cameras. The world camera provides an image of what is available to the subjects visual system and is located directly above the nasion on a pair of glasses. The other two cameras are focused on the subjects left and right eyes, providing information about where the pupil is during a frame capture. By combining the 2-dimensional information provided by these cameras, it is possible to estimate the focus of attention on the world camera scene (see Figure 5.2). The world camera collected data at a sampling rate of 30 Hz, while the eye cameras provided data at a 120 Hz sampling rate, providing, on average, 4 samples of eye locations per world camera image.

Typical eye movement measurements include quantification of saccades and fixations. Saccades are measured by the velocity of the eye movements, typically faster than 300 ms, and are used to assist in changing the location of visual fixation. [265]. Visual fixations are defined as a relatively stable state of the eye and typically last between 100 to 500 ms [286, 285]. In this dissertation, temporal measures regarding when saccades occur, along with when and subsequent fixations begin are utilized to provide insight into the perceptual learning of a sequence [287, 265].

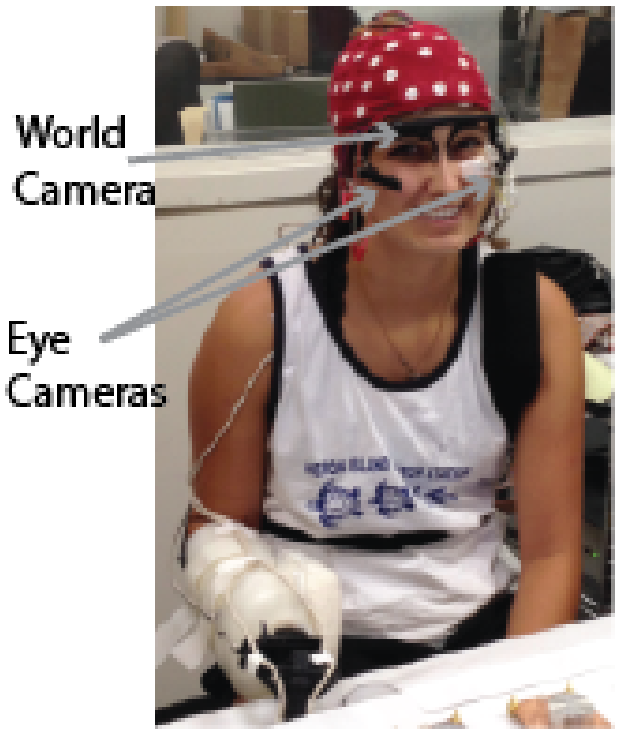


Figure 5.2: **Pupil Labs Eye-tracker.** Eye-tracking system used in specific Aim 3. The world camera is located on the top of the glasses, while an eye camera is located on the side of both eyes.

5.3.3 Kinematic measurements

In addition to utilizing eye-tracking for visual perceptual changes, specific Aim 3 collected three-dimensional hand and should movement information with an electromagnetic motion capture system. Data were sampled at the default system parameters with a sampling rate of 1 KHz. Each subject shoulder size and location, along with arm length were calibrated to provide an individual tracking of movements. Sensor calibration was completed using the recommended protocol by Innovative Sports, Inc. Raw data collected provides the x, y and z-coordinates of each of three sensors throughout the completion of one trial which included both a reach-and-grasp phase and a transport phase. One sensor was placed on the surface of the skin overlaying the right shoulder blade, one was placed on the fixed jaw of the FAMS device or the thumb of the right hand, and the third sensor was placed on the moveable jaw of the FAMS device of the index finder of the right hand. Initial preprocessing was conducted by manufacturer software and provided information regarding velocity magnitude and aperture distances during each movement. Additional analy-

sis regarding movement parameters were conducted using MATLAB software (The MathWorks, Natick, MA). Kinematic measurements of interest included peak velocity and variability, RMSE of reach path, directional error, peak aperture, and shoulder displacement in the lateral direction. Multiple studies have shown the value of these measures in tracking the quality of reaching movements in both healthy and diseased states [288, 289, 290, 291, 292].

5.4 Materials and Methods:

5.4.1 Subjects:

Twenty-four right-handed (age 18-27, 12 females), neurologically healthy adults were recruited to participate in the study. Two upper limb amputees (aged 22 and 55 years of age, both male), were additionally recruited. All subjects provided written, informed consent, and the Georgia Institute of Technology Institutional Review Board approved all methods. Subjects completed an Edinburgh Handedness Inventory [205] to assess the level of handedness along with a short questionnaire regarding any previous musical training they had received. Only subjects with a handedness score greater than 0.6 (indicating right hand dominance), and less than 3 years formal musical training, were included in the study. Amputees also completed the Trinity Amputation and Prosthesis Experience Scale (TAPES) questionnaire [293]. One FAMS subject stopped the experiment after 3 blocks due to fatigue, so only eleven FAMS subjects were included in analysis. Additionally, one amputee subject stopped the experiment after 6 blocks due to fatigue, therefore amputee results (AMP) are included in graphical comparisons for visual identification of potential trends, but were not included in statistical analysis.

5.4.2 Experimental Apparatus:

All subjects experienced the same reach-and-grasp task utilizing aluminum discs (3.5 cm in diameter and 1 cm in height). Each disc had a conductive fabric attached to the base in order to provide an electrical connection for identification of when the disc was picked up from or placed onto one of five targets on the Arduino-based workspace (see Figure 5.3(a)). Each target consisted of a printed

circuit board (PCB), 6.5 cm wide and 10.0 cm long, separated by 4.5 cm. Each PCB contained a circuit which controlled an RGB LED to indicate the current disc to be picked up. Each target board contained three circular circuits which allowed for recording the relative placement of each disc at the end of transport. The Arduino-based workspace also allowed for simultaneous TTL pulses to be sent to a customized MATLAB program for behavioral times (see Behavior section), the eye-tracking recording system (see Gaze Behavior section), the Neuroscan EEG recording system (see EEG section), and the motion capture recording system (see Kinematics section).

5.4.3 Experimental Paradigm:

Healthy subjects were randomly placed in one of two groups: an intact, control (CONT) group and an experimental group, utilizing our fictive amputee model system (FAMS) prosthetic (see Figure 5.1). Amputees were analyzed as a separate, third experimental group (AMPU).

The experiment consisted of 16 blocks total, with one block containing 35 trials, resulting in a total of 560 trials. A trial started with the subject resting his/her right hand on the home base button. After a 500 ms delay, the subject performed a reach-and-grasp to the target disc indicated by a red LED on the circuit board. The subject then transported and placed the disc on the empty circuit board, and returned to the home base button. This movement cycle constituted one trial. Completion of a block (35 trials) was followed by a 45-second rest break indicated by all five red LEDs lighting up simultaneously. Subjects were given a warning signal 3-seconds prior to the end of the rest break by having all LED lights changing to a green color. The first block consisted of 35 random movements and was utilized to establish each subjects baseline performance. The remaining 15 blocks consisted of five repetitions of a 7-element repeating sequence of movements (see Figure 5.3(c).) All subjects received a brief practice session consisting of five trials prior to starting the experiment. After the practice session, subjects were informed that the goal of the experiment was to move the designated disc to the open space as quickly and accurately as possible. After completion of all 16 blocks, subjects were asked questions regarding the recognition of a sequence, asked to recreate the sequence to the best of their recollection, and additional ques-

tions regarding aspects which led them to the recognition of a pattern and any methods utilized to learn the pattern. Subjects able to recreate the sequence with 100% accuracy were classified as explicit (EXP), while those unable to replicate the sequence accurately were classified as non-explicit (NOEXP).

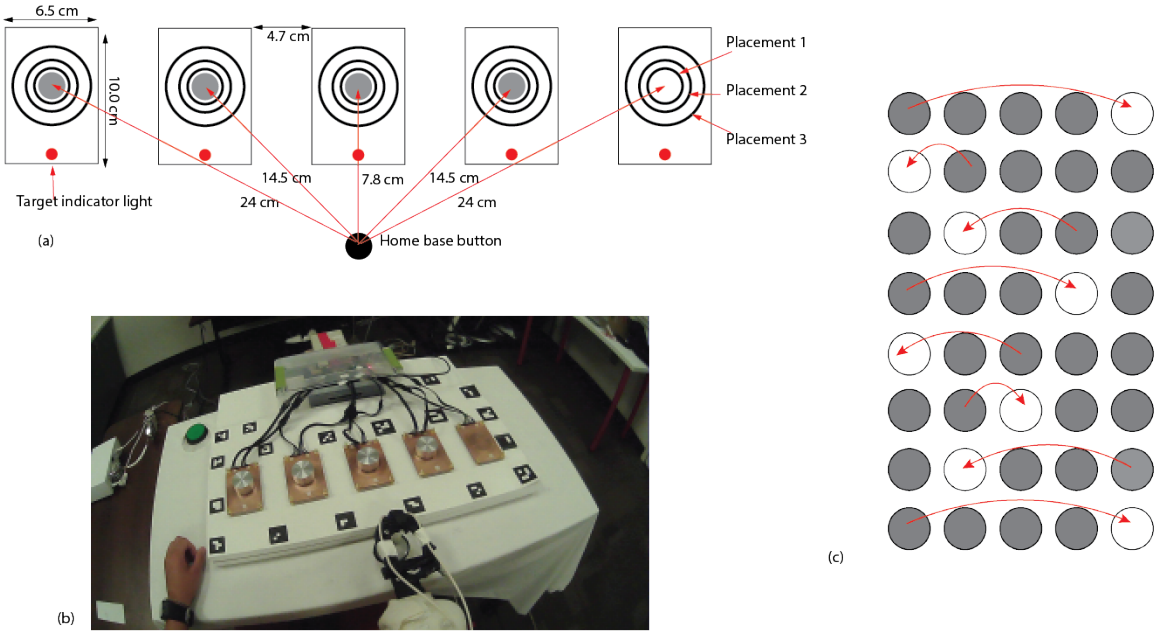


Figure 5.3: **Experimental setup.** (a) Dimensions of, and spacing between, target circuits. Circuit layout allowed for the quantification of three placement locations as a measure of accuracy. The LED provided a visual indication of which disc to pick up, upon which subject was to transfer the disc to the open space. (b) Photograph of experimental setup showing a FAMS subject. (c) 7-element repeating sequence experienced by subjects for a total of 75 repetitions.

5.4.4 Behavioral Data Collection

A customized MATLAB program was connected to the Arduino via a USB port, reading the timings as recorded by the workspace circuitry. Specifically, times received by MATLAB for each trial included: (1) time of red LED onset, (2) time when hand/prosthetic was lifted off home base (HB) button, (3) time when indicated disc was picked up, (4) time when disc was placed on the empty target, and (5) time when the subject pressed the HB button. In addition to the times, the Arduino delivered a value indicating where the disc was placed: 1=disc placed within the inner

circle, 2=disc placed in middle circle, 3=disc placed on outer circle. These times were then utilized to calculate the behavioral measurements of interest: movement onset (MO), movement time (MT), reaction time (RT), transport time (TR), return home (RH), and total time (TT). Figure 5.4 demonstrates the experimental paradigm and measures for each behavioral variable.

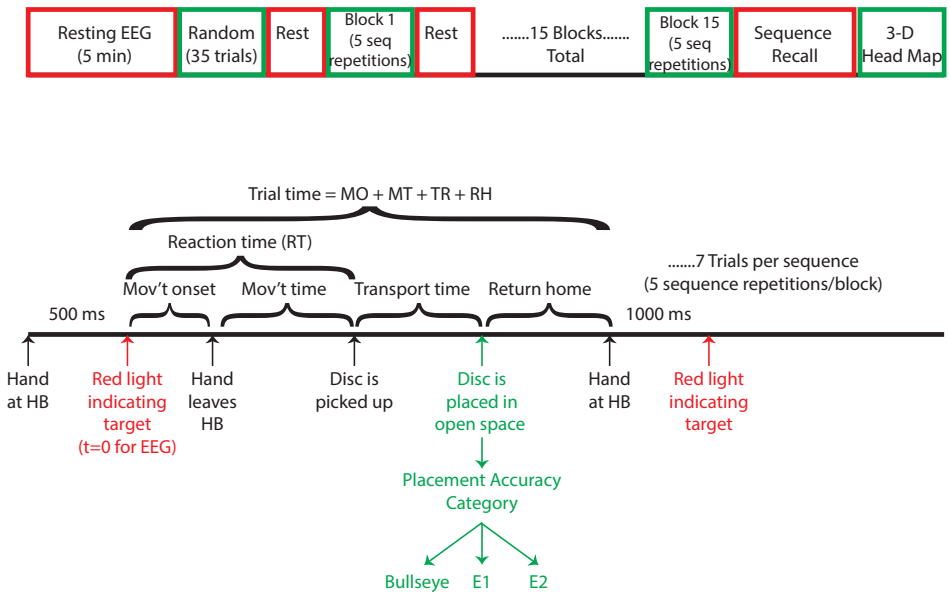


Figure 5.4: **Data Collection.** (a) Experimental paradigm design. (b) Data collection provided by Arduino-based circuit for one trial. Impulses from stimulus onset and movement onset were sent to the EEG recording for epoching. Impulses from movement onset, disc pick up, disc placement and hand pressing HB were sent to Motion Monitor to allow for kinematic analysis of reach-and-grasp and transport phases separately.

Movement onset (MO) = Leaves HB Stimulus onset (SO) Movement time (MT) = Disc picked up Leaves HB Reaction time (RT) = MO + MT Transport time (TR) = Disc placed Disc picked up Return home (RH) = Pushes HB button Disc placed Trial time (TT) = MO + MT + TR + RH Placement = (1) Bullseye, (2) Middle circle, (3) Outer circle

5.4.5 Kinematic measurements

Kinematic measurements of interest for specific Aim 3 were limited to the reach-and-grasp phase of each trial, specifically those related to movement quality changes over time. The reach-and-grasp phase was defined as the time from movement onset to the time the disc was picked up. The measures focused on included: (1) peak velocity of reach phase, (2) coefficient of variation (CV) of reach velocity, (3) Root-mean-square-error (RMSE) of reach path, (4) peak aperture, (5) lateral trunk movement, (6) directional error.

Peak velocity provides a measure of movement vigor, which has been shown to increase with explicit awareness. To examine the potential impact of this increased speed on the speed-accuracy-tradeoff, velocity CV and RMSE were calculated as measures of movement accuracy. Lateral trunk movement was also examined, as this parameter has been shown to be altered in prosthetic use as compensatory behaviors [294]. To identify the presence of anticipatory movements reflective of sequence-related predictions, directional errors were calculated. Both the RMSE and directional error measures are calculated as deviations from a predicted straight line path in the x-y coordinate plane. To address the variation in disc location throughout the experiment, the predicted straight line trajectory for each trial was calculated as the line between the point of the thumb/fixed jaw at movement onset to the point of the thumb/fixed jaw at disc pick up. RMSE was calculated as the thumb/fixed jaw variation from this path in the y-direction throughout the reach movement. Directional error was determined by comparing the initial reach trajectory angle from the horizontal plane of the first 15% of the movement path to the predicted straight-line trajectory angle from the horizontal plane (see Figure 5.5).

5.4.6 Gaze analysis

Gaze analysis was conducted utilizing the Surface Marker detection plugin provided for Pupil Labs. This program allowed for offline identification of the workspace through utilization of mark-

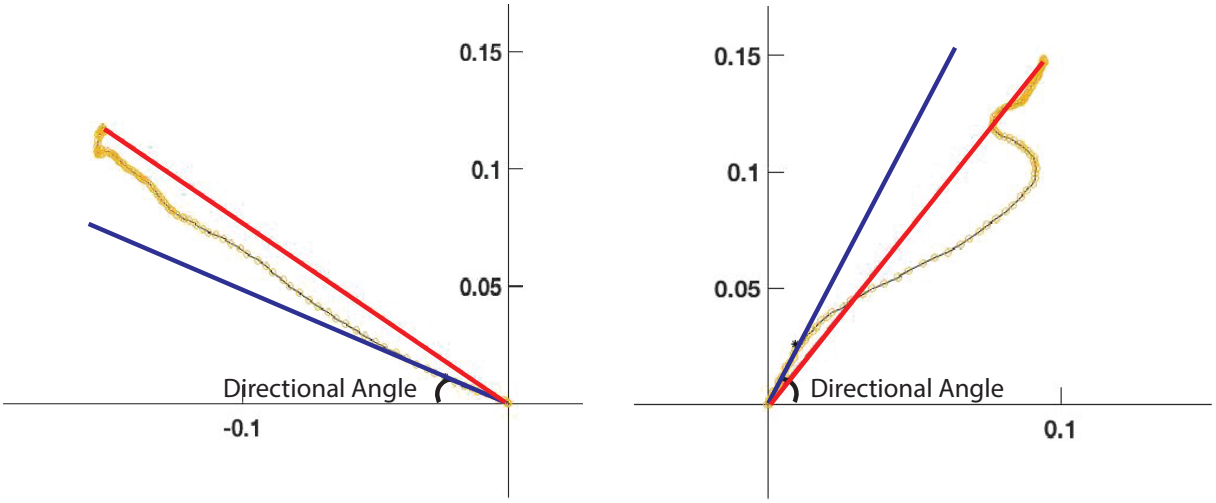


Figure 5.5: **Kinematic Analysis.** Sample subject reach path. RMSE was calculated as the deviation in the y-direction from the perfect reach path (red). Directional error was calculated as the differences in angles from the horizontal to the perfect reach path and the first 15% of the movement path.

ers which were placed on the workspace prior to conducting the experiment. The markers were then calibrated to both the world camera and eye camera videos, allowing the identified areas of interest (AOI) to be tracked with the subjects head movement. Two gaze parameters were calculated utilizing Surface Marker detection: saccade onset and gaze onset. Saccade onset AOI included the entire workspace from the left to the right edge of the wooden block (60 x 30 cm). This AOI provided information regarding (x,y) gaze coordinates over the workspace to identify when a subject initiated a saccade to change fixation location. Saccade initiation was calculated utilizing a method introduced previously in our lab [295]. A sliding window of 100 ms was used to identify the mean current (x,y) position of gaze. If the next identified gaze position exceed $\pm$ 2SD of the mean current gaze position, a saccade was identified. The latency of the first, initial saccade post-stimulus onset and the total number of saccades during the reach profile were calculated. Due to the ability of subjects to freely move their eyes during the experiment, there were times when the gaze was on the target upon stimulus onset, resulting in no initial saccade. Additionally, to prevent inclusion of exploratory gaze patterns, no saccades initiated pre-stimulus were included in the analysis.

The second gaze parameter examined was latency of gaze fixation onset to the current target

of interest. Five AOIs were drawn on the world camera video using the Surface Marker plugin to include $\pm$ 2 cm around each circuit board. This provided a range which would detect fixations which utilized extrafoveal information, while preventing overlap between targets. Fixations were defined as gaze patterns in which the gaze location remained in the target of interest for a minimum of 120 ms.

5.4.7 EEG Recording and Pre-Processing

EEG recording and pre-processing utilized a similar protocol as in Specific Aims 1 and 2, with the exception of the epoch length. Due to the extended nature of the task, epochs were made up to 1000 ms post-stimulus onset. An LED pulse from the Arduino workspace provided a pulse indicating both stimulus onset and movement onset for each trial. The raw, continuous EEG data was imported into MATLABs EEGLAB and epoched from 100 ms pre-stimulus onset to 1000 ms post-stimulus onset for VEP analysis. Saccade latencies were imported as events into each individuals EEG data to allow for neural sorting according to saccade onset latencies.

Epoched data was pruned utilizing ICA component analysis. Due to the larger movements required in the three-dimensional task, an average of 25 of 58 components were removed. In addition, individual data epochs with extreme voltage values greater than +/- 100 uV were removed, with an average rejection of 8 epochs per subject. Following artifact removal, datasets were combined for each condition based on awareness, resulting in a total of 6 possible groups: CONT_EXP, CONT_NOEXP, FAMS_EXP, FAMS_NOEXP, AMP_EXP, AMP_NOEXP.

For individual data analysis, epochs were segmented into separate datasets for each block. Dipole fitting was conducted and all blocks loaded into EEGLABs STUDY structure for individual area amplitude analysis. Due to the increase in time between stimulus onset and movement onset, compared to Specific Aims 1 and 2, the time windows utilized for area amplitude calculations were widened (EarlyN1:50-200 ms, LateN1:130-200 ms, P2:150-300 ms, P3:250-500 ms). Calculations of area amplitude were then conducted over the same electrodes, and in the same manner, as used for Specific Aims 1 and 2.

5.4.8 EEG Movement onset and saccade onset sorted VEP image

Combined EEG datasets for each group was examined by sorting individual trials based on both movement onset and saccade onset latencies separately utilizing the VEP image plugin for EEGLAB. This method provides a means for comparing individual trial VEP data which may vary significantly in the timing of visual or motor aspect. Recent studies have shown this method valuable in revealing neural changes related to the sorted variable which would otherwise be masked when examining data locked to the stimulus onset [296, 295].

5.4.9 Determination of baseline for individualized threshold

Due to the availability of movement onset times, a different method for identifying the onset of explicit awareness was utilized. Previous studies have shown that a visual processing time of 100 ms is required for stimuli recognition, with additional delays involved in movement selection [297, 298]. Therefore it was assumed that any movement onset faster than 110 ms was reflective of anticipatory movements. Some subjects did demonstrate a very impulsive bounce off of the home base button, recording a movement onset time of more than 400 ms prior to movement onset. Therefore, any movement onset times faster than 400 ms prior to movement onset were discarded from analysis. As with Aims 1 and 2, the requirement of having movement onset times with the upper confidence interval below 110 ms for two consecutive sequence repetitions was required for classification of EXP behavior. The block at which such EXP behavior occurred was utilized for neurobehavioral correlations with the area amplitude measures, while for subjects classified as NOEXP, the block of the lowest z-score was utilized.

5.4.10 Statistical Analysis

Due to the limited number of AMP subjects, with only one completing all 15 blocks, AMP subject data is provided for visual reference only, and not included in statistical comparisons. For behavioral measures, a 2 x 2 ANOVA was conducted with between factors of awareness (2 levels: EXP and NOEXP) and group (2 levels: CONT and FAMS). For repeated measures statistical

comparisons, to address the issue of unequal sample sizes between groups, a linear mixed model approach was utilized as in Aim 2. Models structures utilized are described below. Three levels of time were used, with the BOI being identified as the block of explicit behavior for EXP subjects and the block of lowest z-score performance for NOEXP subjects. For model comparisons which revealed significant effects, a subsequent post hoc pairwise comparison was conducted with single-step Bonferonni corrections for multiple comparisons. Adjusted significance levels are reported at $p<0.05$ for both model and pairwise comparisons.

Model 0 (M0) = Repeated measures (RM) with random effect of subject

Model 1 (M1) = RM with fixed effect of group (2 levels: CONT and FAMS) Model 2 (M2) = RM with fixed effect of awareness (2 levels: EXP and NOEXP)

Model 3 (M3) = RM with fixed effect of time (3 levels: Random, BOI, Block 15)

Model 4 (M4) = RM with interaction between fixed effects of group and awareness (2 x 2)

Model 5 (M5) = RM with interaction between fixed effects of group, awareness and time (2 x 2 x 3)

5.5 Results

5.5.1 Explicit Recall

Five of the twelve CONT subjects and four of the twelve FAMS were able to recall the 7-element sequence to 100% accuracy, and were classified as EXP, while those unable to recall to 100% accuracy were classified as NOEXP (see Figure 5.6(a)). There was a near significant effect for timing of explicit behavior ($F(7,1)=4.054$, $p=0.08$) with explicit behavior for CONT_EXP appearing by Block 9 ± 3 and FAMS_EXP subjects by Block 12 ± 1. As shown in Figure 5.6(b), there was a significant main effect of group ($F(14,2)=3.719$, $p=0.05$) for level of recall within the NOEXP awareness group with CONT subjects recalling a larger percentage of the sequence compared to AMP subjects ($p=0.01$). There was no significant difference between the NOEXP_FAMS and AMP subjects.

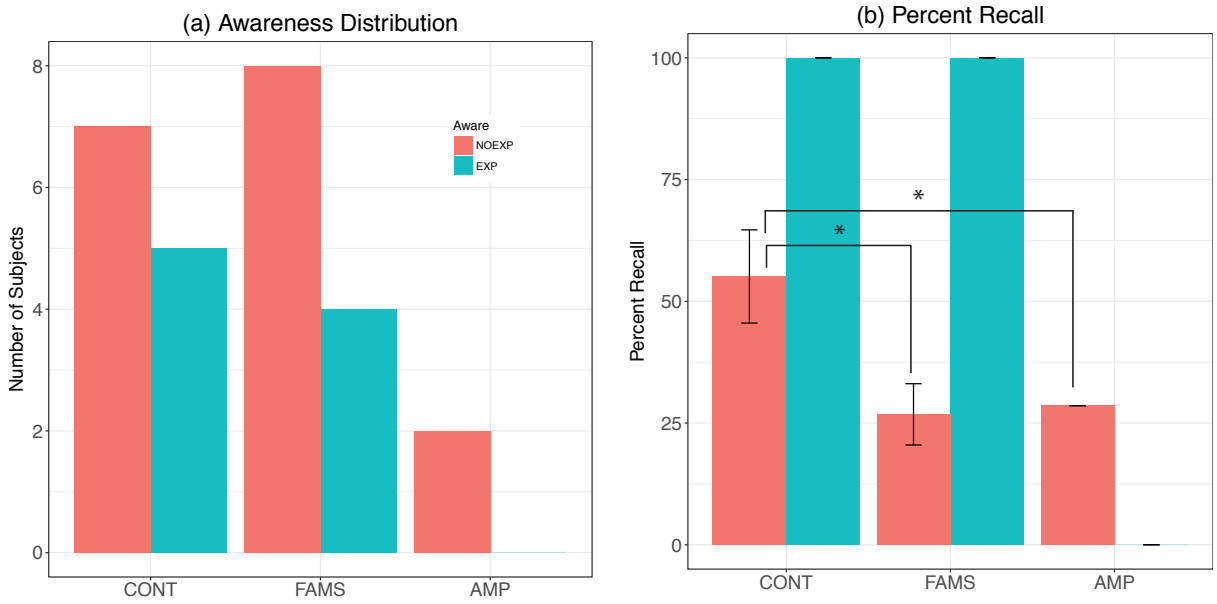


Figure 5.6: **Recall of Sequence.** (a) 5 of 12 CONT subjects and 4 of 12 FAMS subjects demonstrated 100% recall of the sequence (b) For NOEXP subjects, the CONT subjects demonstrated a significantly greater recall level than FAMS or AMP subjects.

5.5.2 Movement Onset Threshold Reliability

Identification of timing of explicit awareness development was identified as the block in which subjects demonstrated movement onset times with an upper confidence interval of less than 110 ms for two consecutive sequence repetitions. Figure 5.7 shows sample movement onset times for subjects in both groups and awareness conditions.

Utilization of the movement onset threshold resulted in eight of the nine EXP subjects being accurately classified as EXP, with one false negative. Thirteen of the sixteen NOEXP subjects were accurately classified as being NOEXP. This provided a sensitivity of 72.7% and a specificity

of 92.8% in accurately classifying a subject's level of awareness. Based on this level of reliability, neural examination of the facilitative network was conducted as in Aims 1 and 2.

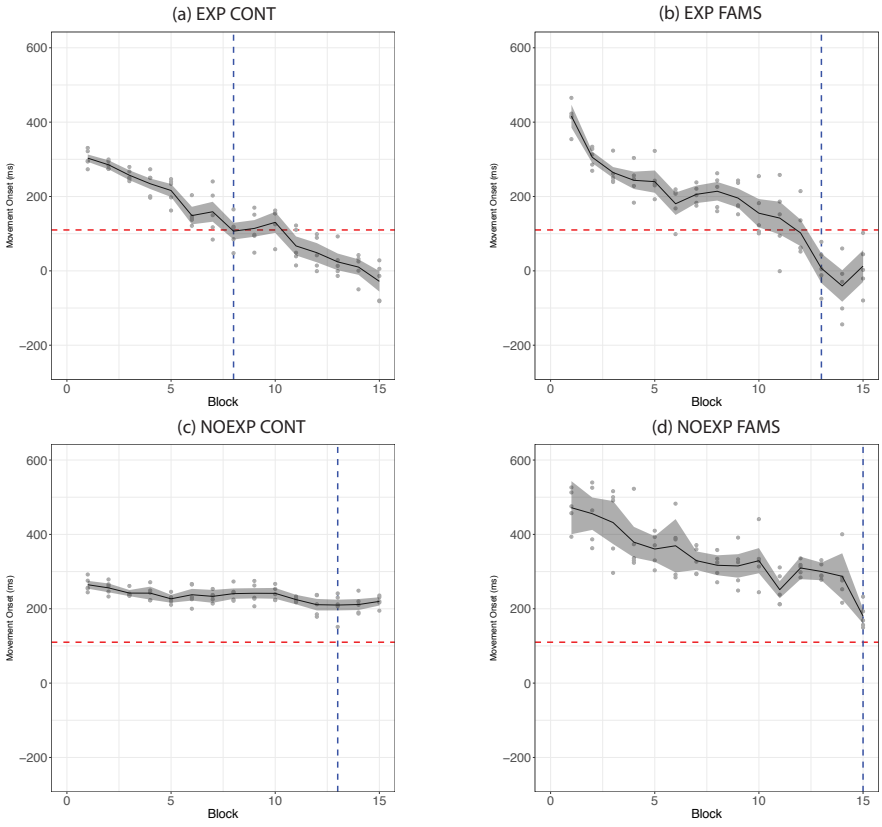


Figure 5.7: **Sample Movement Onset Data.** Sample movement onset times for EXP and NOEXP subjects. For EXP subjects, the blue vertical line indicates when the upper confidence interval was less than 110 ms for two consecutive sequence repetitions. For NOEXP subjects, the blue vertical line indicates the block when the fastest upper confidence interval was achieved (a) EXP CONT subject demonstrating explicit behavior Block 5. (b) EXP FAMS subject demonstrating explicit behavior Block 13. (c) NOEXP CONT subject with fastest z-score at Block 13. (d) NOEXP FAMS subject with fastest z-score at Block 14.

5.5.3 Neurobehavioral Correlations:

Correlations between timing of behavior predictive of awareness based on movement onset threshold demonstrate significant correlations for EXP subjects over both the PCC (rho=0.82, p=0.007)

and SMA (rho=0.93, p<0.001). No significant correlation was noted over the RPcun (rho=0.47, p=0.21), LPcun (rho=0.59, p=0.093) and ACC (rho=0.55, p=0.13). NOEXP subjects only demonstrated significant neurobehavioral correlations over the PCC (rho=0.64, p=0.03). Figure 5.8 summarizes the neurobehavioral correlations for both EXP and NOEXP subjects.

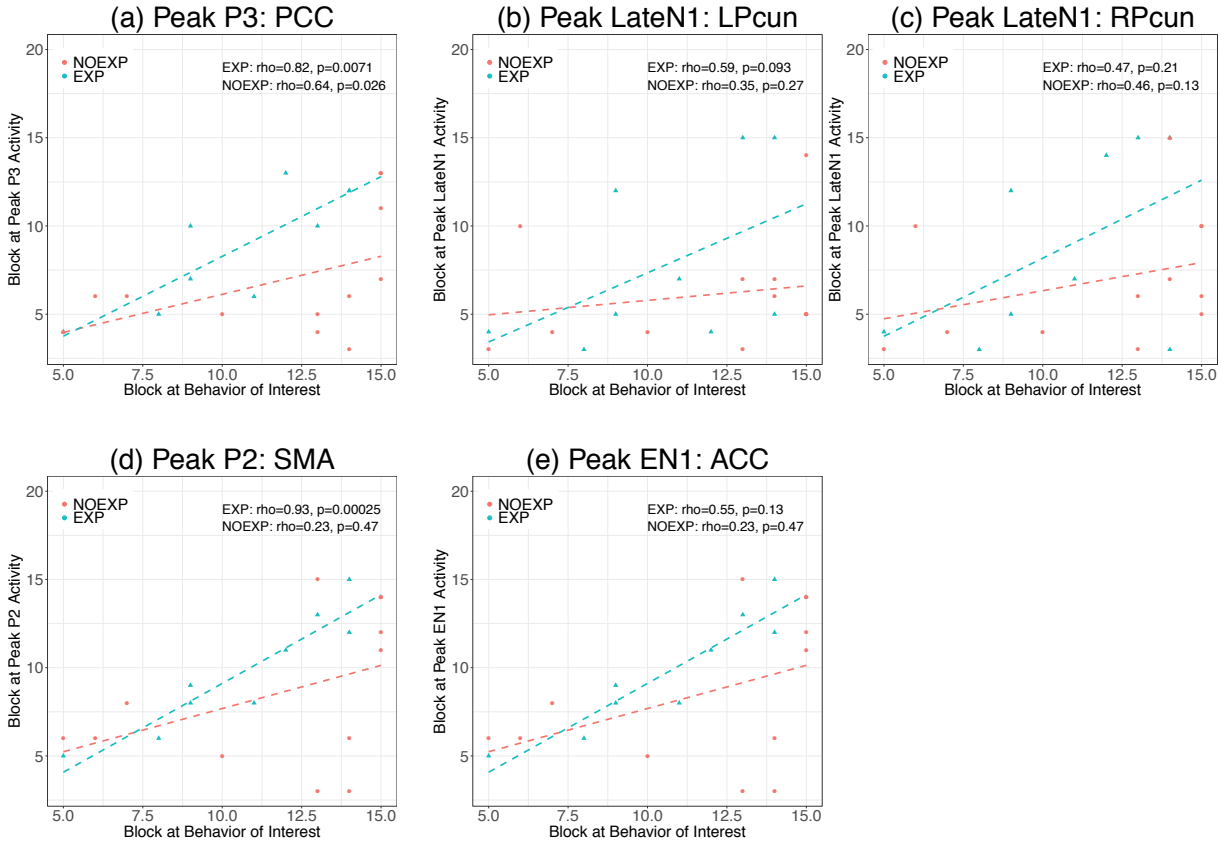


Figure 5.8: **Neurobehavioral Correlations.** Correlations between timing of EXP behavior, or fastest z-score, and peak neural activity over regions implicated in facilitative network presented in Aims 1 and 2.

5.5.4 Behavioral, Gaze and Kinematic Correlates to Explicit Awareness Development:

Working Memory. As was seen in Aim 2, there was a near significant main effect for awareness in the working memory scores (F(24,1=3.086, p=0.09), with NOEXP subjects performing much slower on the 2-back task (see Figure 5.9(a)). Closer examination of 2-back score distributions,

revealed a wider distribution of 2-back scores for the FAMS_NOEXP group compared to all other groups (see Figure 5.9(b)).

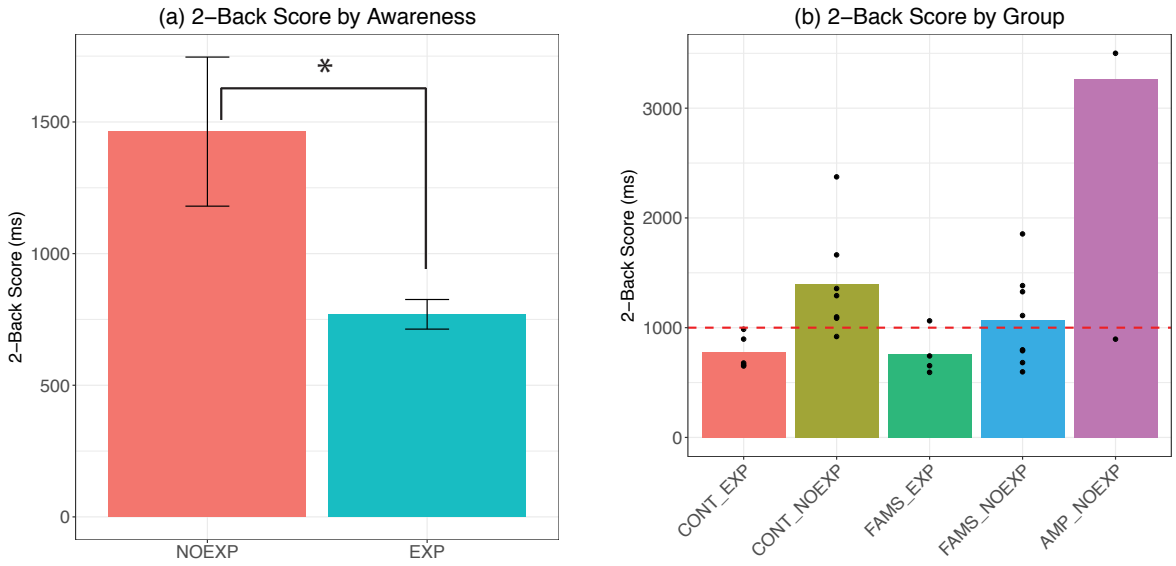


Figure 5.9: **Working Memory.** (a) EXP subjects demonstrate a significantly faster 2-back score. (b) Distribution of n-back scores for subjects across group and awareness level. Notice the low number of NOEXP_CONT subjects scoring below 1000 ms, compared to the number of NOEXP_FAMS subjects scoring below 1000 ms.

Behavioral and Kinematic Changes Over Time: Speed, Accuracy and Gaze

Behavioral and kinematic measures of speed included movement onset (Table 5.1), movement time (Table 5.2), reaction time (Table 5.3), and peak velocity (Table 5.4) during reach. Figure 5.10 summarizes these measures of speed for at three specific points in the experiment: (1) Random block, (2) block of explicit behavior or fastest z-score performance, (3) Block 15 (last block). Table 5.5 shows the linear mixed model results for all four speed variables. For movement onset, there was no significant main effect of group ($p=0.53$), a significant main effect of awareness ($p=0.009$), and a significant main effect of block ($p<0.001$). Significant interaction effects were identified for Awareness x Group ($p=0.09$) and Awareness x Group x Block ($p<0.001$). Note the significantly faster MO for EXP_CONT and EXP_FAMS compared to NOEXP_CONT and NO-

EXP_FAMS at BOI and B15 (Figure 5.10(a)). Movement time measures demonstrated a significant main effect of Group ($p<0.001$), no significant main effect of Awareness ($p=0.12$), and a significant main effect of Block ($p<0.001$). No significant interaction effect was identified for Awareness x Group ($p=0.12$), but a significant interaction effect for Awareness x Group x Block ($p<0.001$) was noted. FAMS subjects demonstrated significantly slower MT's compared to CONT subjects at Random. While all groups demonstrated a decrease in movement time over the blocks, it was noted that by B15, EXP_FAMS subjects had reached a MT similar to NOEXP_CONT subjects. NOEXP_FAMS subjects retained a significantly slower MT compared to NOEXP_CONT subjects (Figure 5.10(b)). Reaction time measures revealed significant main effects of Group ($p<0.001$), Awareness ($p=0.04$), and Block ($p<0.001$), along with significant interaction effects ($p=0.04$ and $p<0.001$). As with MT, FAMS subjects demonstrated significantly slower RT's compared to CONT. By B15, it was noted that EXP_FAMS subjects demonstrated significantly faster RT's compared to NOEXP_CONT subjects (Figure 5.10(c)). Similar to RT, peak velocity showed significant main effects of Group ($p=0.04$), Awareness ($p=0.01$), and Block ($p<0.001$), along with significant interaction effects ($p=0.003$ and $p<0.001$). EXP_CONT subjects had a significantly faster peak velocity at Random compared to all other groups. By B15, EXP_FAMS subjects demonstrated a peak velocity similar to EXP_CONT subjects (Figure 5.10(d)).

Table 5.1: **Movement onset (ms) for groups and awareness over blocks.**

Block	**EXP_CONT**	**NOEXP_CONT**	**EXP_FAMS**	**NOEXP_FAMS**	**NOEXP_AMP**
$Random$	283.0 $\pm$ 79.3	404.08 $\pm$ 205.21	188.31 $\pm$ 294.89	354.86 $\pm$ 142.20	414.37 $\pm$ 121.90
BOI	121.87 $\pm$ 75.5	159.00 $\pm$ 100.08	94.31 $\pm$ 164.67	263.55 $\pm$ 420.27	173.27 $\pm$ 246.36
$B15$	79.60 $\pm$ 99.36	203.36 $\pm$ 110.16	-2.21 $\pm$ 154.88	110.29 $\pm$ 126.34	-3.00 $\pm$ 218.76

Kinematic measures of accuracy included velocity CV (5.8), directional error (5.6, and lateral trunk movement (5.7). Figure 5.11 shows these three measures of accuracy for at three specific points in the experiment: (1) Random block, (2) block of explicit behavior or fastest z-score performance, (3) Block 15 (last block). Table 5.9 shows the linear mixed model results for all

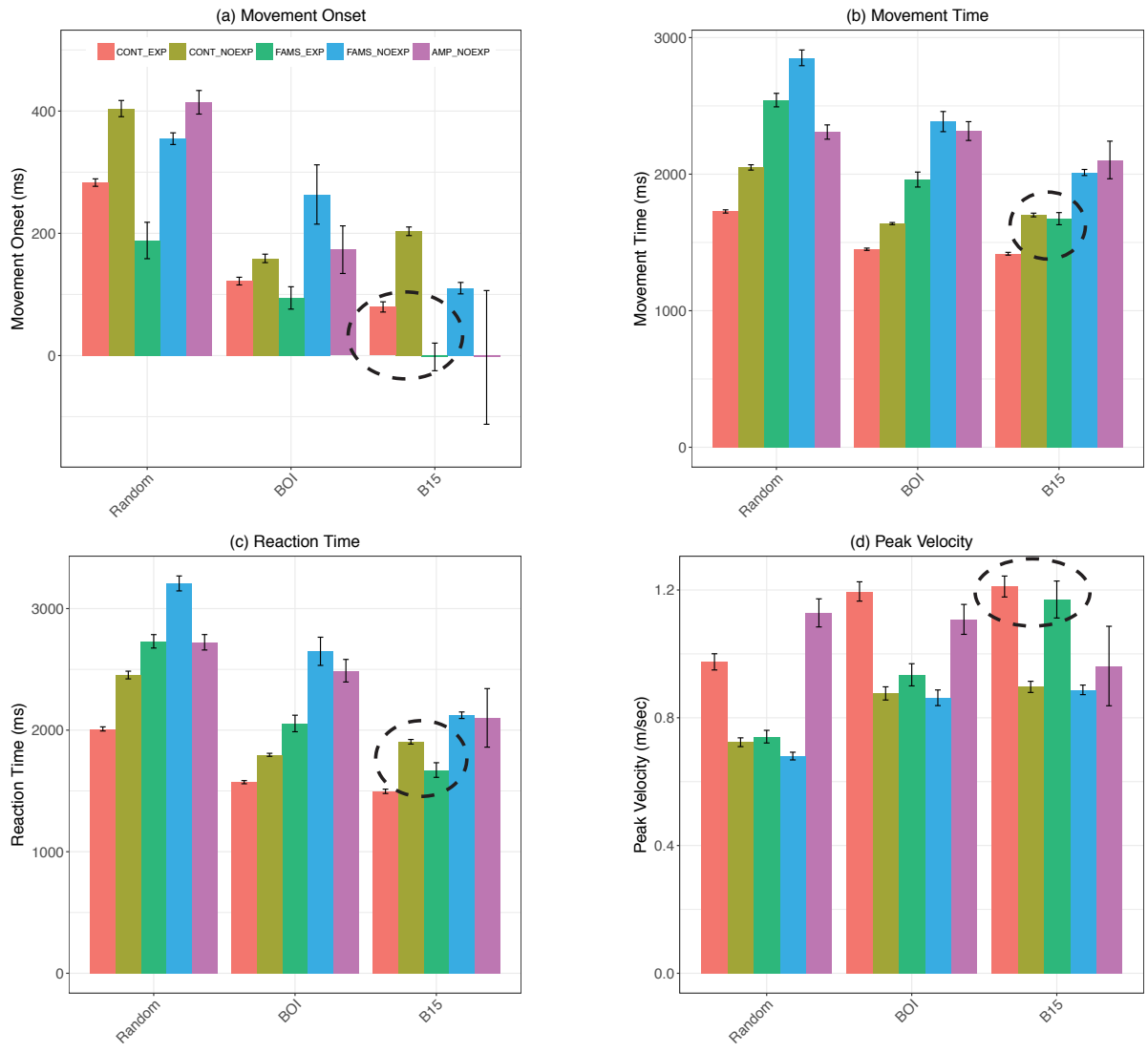


Figure 5.10: **Behavioral and kinematic measures of speed during Random block.** (a) Movement onset (MO). Both EXP_CONT and EXP_FAMS subjects demonstrate MO times faster than 110 ms by B15 indicating anticipatory movements. (b) Movement time (MT). EXP_FAMS subjects reached a MT similar to NOEXP_CONT subjects by B15. (c) Reaction time (RT). EXP_FAMS subjects show a significantly faster RT than NOEXP_CONT by B15. (d) Peak velocity (PV) during reach. EXP_FAMS subjects achieve a PV similar to EXP_CONT by B15.

three accuracy variables. Directional error showed no significant main effect of Group ($p=0.23$) or Awareness ($p=0.64$), but a significant main effect of Block ($p=0.007$). Interaction effects revealed no significant effect for Group x Awareness was found ($p=0.68$), but a significant effect of Group x Awareness x Block ($p<0.001$). EXP_CONT subjects showed significantly greater di-

Table 5.2: **Movement times (ms) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	1727.08 $\pm$ 152.50	2049.38 $\pm$ 302.06	2542.46 $\pm$ 489.69	2852.08 $\pm$ 855.27	2308.50 $\pm$ 330.86
BOI	1450.12 $\pm$ 99.09	1638.04 $\pm$ 112.35	1960.56 $\pm$ 493.08	2385.01 $\pm$ 638.64	2315.67 $\pm$ 435.45
$B15$	1417.26 $\pm$ 121.18	1701.00 $\pm$ 210.80	1674.085 $\pm$ 305.33	2012.37 $\pm$ 299.20	2104.00 $\pm$ 275.36

Table 5.3: **Reaction times (ms) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	2010.11 $\pm$ 221.50	2453.46 $\pm$ 490.04	2730.76 $\pm$ 547.26	3206.94 $\pm$ 913.14	2722.87 $\pm$ 402.10
BOI	1571.99 $\pm$ 155.49	1797.03 $\pm$ 179.93	2054.86 $\pm$ 612.24	2648.56 $\pm$ 1004.16	2488.95 $\pm$ 586.90
$B15$	1496.85 $\pm$ 213.83	1904.36 $\pm$ 294.43	1671.87 $\pm$ 412.86	2122.66 $\pm$ 362.74	2101.00 $\pm$ 481.65

Table 5.4: **Peak velocity (m/s) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	0.98 $\pm$ 0.33	0.72 $\pm$ 0.21	0.74 $\pm$ 0.19	0.68 $\pm$ 0.18	1.13 $\pm$ 0.28
BOI	1.20 $\pm$ 0.36	0.88 $\pm$ 0.30	0.93 $\pm$ 0.31	0.86 $\pm$ 0.21	1.11 $\pm$ 0.30
$B15$	1.21 $\pm$ 0.39	0.90 $\pm$ 0.27	1.17 $\pm$ 0.39	0.89 $\pm$ 0.20	0.96 $\pm$ 0.25

rectional error compared to NOEXP_CONT subjects at Random, with directional error decreasing over time for EXP_CONT subjects, but increasing for NOEXP_CONT subjects (5.11(a)). Lateral trunk movement revealed a significant main effect of Group ($p<0.001$), no significant main effect of Awareness ($p=0.97$), and a significant main effect of Block ($p<0.001$). No significant interaction effect for Group x Awareness was found ($p=0.30$), but a significant interaction effect for Group x Awareness x Block was noted ($p<0.001$). FAMS subjects showed greater lateral trunk movement compared to CONT subjects at Random. By B15, a significant difference between EXP_FAMS and NOEXP_FAMS subjects was noted, with EXP_FAMS subjects increasing the trunk movement, while NOEXP_FAMS subjects decreased lateral trunk movement (5.11(b)). Velocity variability (CV) showed a significant main effect of Group ($p<0.001$), no significant main effect of Awareness ($p=0.26$), and a significant main effect of Block ($p<0.001$). Interaction effects revealed no

Table 5.5: **Behavioral and kinematic measures of speed over blocks between groups and awareness.**

Model Test	Analysis	df	MO	MT	RT	PV
$M0$	Repeated measures	3				
$M0vs.M1$	Main Effect of Group	4	p=0.53	**p<0.001**	**p<0.001**	**p=0.04**
$M0vs.M2$	Main Effect of Awareness	4	**p=0.009**	p=0.12	**p=0.04**	**p=0.01**
$M0vs.M3$	Main Effect of Block	5	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
$M1vs.M4$	Interaction Effect of Awareness & Group	6	**p=0.02**	p=0.12	**p=0.04**	**p=0.003**
$M4vs.M5$	Interaction Effect of Awareness, Group, and Block	14	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**

significant effect for Group x Awareness (p=0.10), but a significant effect for Group x Awareness x Block (p<0.001). FAMS subjects had a greater velocity variability at Random compared with CONT subjects, with no significant difference between EXP_FAMS and NOEXP_FAMS subjects. By B15, EXP_FAMS subjects demonstrated significantly greater velocity variability compared to NOEXP_FAMS subjects (Figure 5.11(c)).

Table 5.6: **Directional Error (degrees) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	20.10 $\pm$ 15.73	15.83 $\pm$ 16.23	22.21 $\pm$ 24.26	19.65 $\pm$ 18.23	44.77 $\pm$ 56.12
BOI	14.78 $\pm$ 11.83	24.67 $\pm$ 36.39	23.88 $\pm$ 28.82	30.58 $\pm$ 34.39	51.40 $\pm$ 66.89
$B15$	15.02 $\pm$ 14.74	21.40 $\pm$ 30.18	21.03 $\pm$ 12.63	20.22 $\pm$ 24.1	36.05 $\pm$ 35.76

Table 5.7: **Lateral trunk movement (cm) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	1.14 $\pm$ 0.40	1.46 $\pm$ 0.60	5.51 $\pm$ 3.64	4.52 $\pm$ 2.43	3.16 $\pm$ 1.59
BOI	1.03 $\pm$ 0.47	1.28 $\pm$ 0.51	5.28 $\pm$ 2.56	4.69 $\pm$ 2.14	4.17 $\pm$ 2.38
$B15$	0.99 $\pm$ 0.38	1.49 $\pm$ 0.79	6.26 $\pm$ 2.74	3.40 $\pm$ 1.76	6.14 $\pm$ 3.21

Gaze behavioral measures included initial saccade latency post-stimulus (5.10), the number

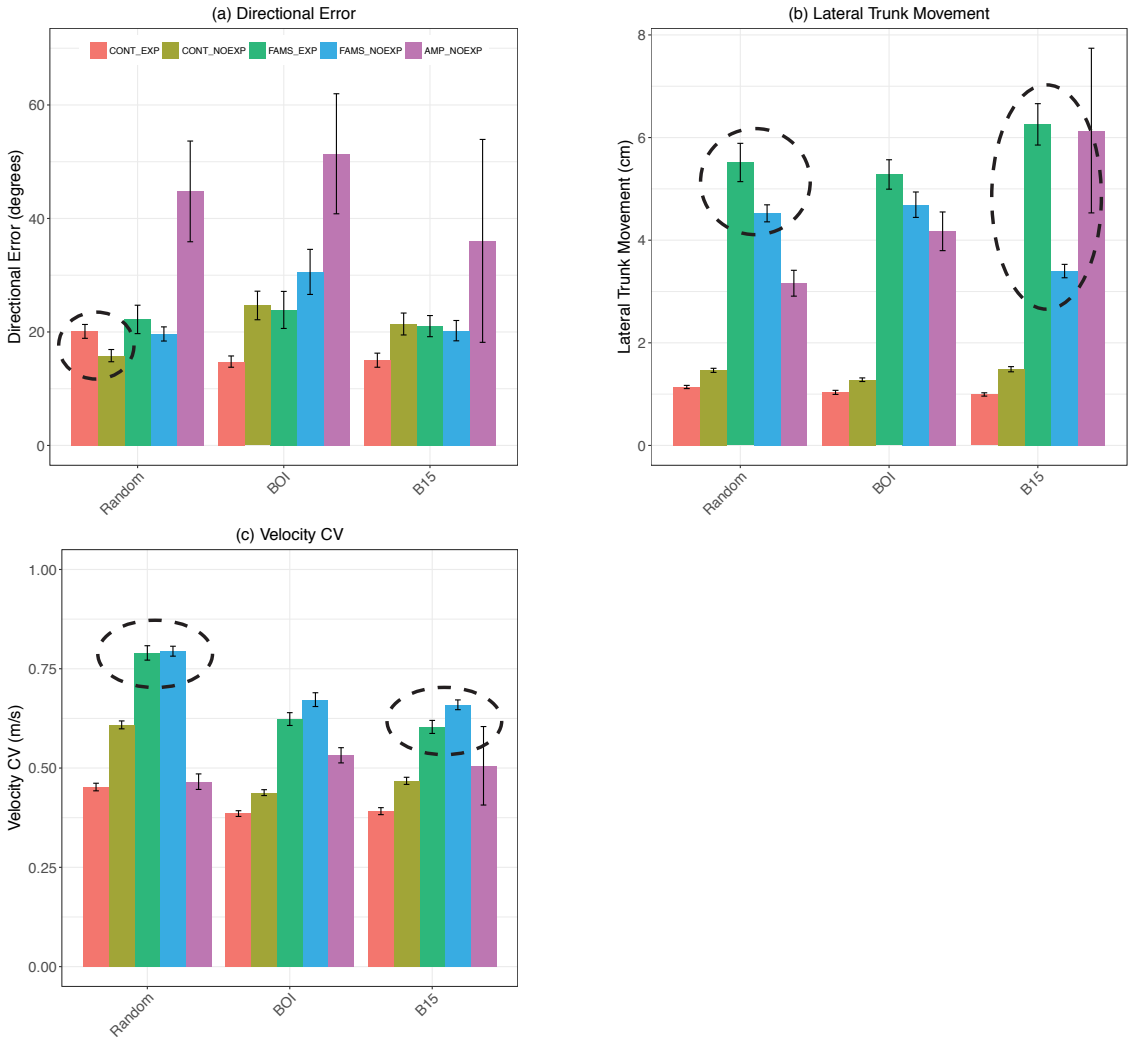


Figure 5.11: **Kinematic measures of accuracy of reach movement during Random block.** (a) Directional error. EXP_CONT subjects demonstrate significantly greater directional error compared to NOEXP_CONT subjects at Random. The degree of directional error is noted to decrease for EXP_CONT by BOI, but increases EXP_CONT subjects,. (b) Lateral trunk movement. FAMS subjects show significantly greater trunk movement compared to CONT subjects throughout the experiment, with EXP_FAMS subjects having more trunk movement than NOEXP_FAMS subjects both at Random and B15. (c) Velocity CV. FAMS subjects demonstrate significantly greater velocity variability than CONT subjects throughout the course of the experiment. While both EXP_FAMS and NOEXP_FAMS subjects show similar levels of variability at Random with variability decreasing over the experiment, EXP_FAMS variability decreases significantly more than NOEXP_FAMS by B15.

of saccades between stimulus onset and picking up of the disc (5.11), and gaze onset latency to the target of interest (5.12). Table 5.13 and Figure 5.12 summarize the gaze behavior measures

Table 5.8: **Velocity coefficient of variation (m/s) for groups and awareness over blocks.**

Block	EXP_CONT	NOEXP_CONT	EXP_FAMS	NOEXP_FAMS	NOEXP_AMP
$Random$	0.45 ± 0.12	0.61 ± 0.15	0.79 ± 0.18	0.79 ± 0.18	0.47 ± 0.12
BOI	0.39 ± 0.09	0.44 ± 0.11	0.62 ± 0.14	0.67 ± 0.15	0.53 ± 0.12
$B15$	0.39 ± 0.10	0.47 ± 0.14	0.60 ± 0.11	0.66 ± 0.16	0.51 ± 0.20

Table 5.9: **Behavioral and kinematic measures of accuracy over blocks between groups and awareness.**

Model Test	Analysis	df	Dir Error	Trunk Movement	Vel CV
$M0$	Repeated measures	3			
$M0vs.M1$	Main Effect of Group	4	p=0.23	**p<0.001**	**p<0.001**
$M0vs.M2$	Main Effect of Awareness	4	p=0.64	p=0.97	p=0.26
$M0vs.M3$	Main Effect of Block	5	**p<0.007**	**p<0.001**	**p<0.001**
$M1vs.M4$	Interaction Effect of Awareness & Group	6	p=0.68	p=0.30	p=0.10
$M4vs.M5$	Interaction Effect of Awareness, Group, and Block	14	**p<0.001**	**p<0.001**	**p<0.001**

and statistical comparisons for the Random block, BOI and B15, while Figure 5.13 shows saccade behavior changes over the course of the entire experiment. One EXP_FAMS subject had unusual gaze behavior resulting in the inability to access fixation or saccade information using the Surface Marker plugin and is, therefore, excluded from statistical analysis for gaze behavior. Saccade onset revealed no significant main effect of Group (p=0.51) or Awareness (p=0.25), but a significant main effect of Block (p<0.001). Interaction effects showed no significant effect of Group x Awareness, but a significant effect of Group x Awareness x Block (p<0.001). All groups with the exception of EXP_FAMS demonstrated a significant decrease in saccade onset over the experiment (Figure 5.12(a)). The number of saccades showed a significant main effect of Group (p=0.002) and Block (p<0.001), but no significant main effect of Awareness (p=0.25). Interaction effects showed no significant effect of Group x Awareness (p=0.93), but a significant effect of Group x

Awareness x Block ($p<0.001$). FAMS subject showed a greater number of saccades at Random compared to CONT subjects. FAMS subjects demonstrated a significant decrease in the number of saccades of the experiment, while CONT subjects showed no significant change in the number of saccades (Figure 5.12(b)). Gaze onset measures showed no significant main effect of Group ($p=0.08$), but a significant main effect of Awareness ($p=<0.001$) and Block ($p<.001$). Interaction effects revealed significant effects for both Group x Awareness ($p<0.001$) and Group x Awareness x Block ($p<0.001$). There was no significant difference between groups at Random. All groups demonstrated a significant decrease in gaze onset latency over blocks, but both EXP_CONT and EXP_FAMS subjects showed significantly greater decreases in latency compared to NOEXP subjects (Figure 5.12(c).

Table 5.10: **Saccade onset (ms) for groups and awareness over blocks.**

Block	**EXP_CONT**	**NOEXP_CONT**	**EXP_FAMS**	**NOEXP_FAMS**	**NOEXP_AMP**
$Random$	97.37 $\pm$ 63.66	131.32 $\pm$ 98.90	109.52 $\pm$ 72.53	117.59 $\pm$ 103.80	102.34 $\pm$ 59.73
BOI	59.50 $\pm$ 46.51	81.22 $\pm$ 54.81	81.76 $\pm$ 77.70	123.00 $\pm$ 196.43	100.96 $\pm$ 59.73
$B15$	68.55 $\pm$ 45.32	89.41 $\pm$ 61.86	78.32 $\pm$ 72.17	70.80 $\pm$ 56.00	Not available

Table 5.11: **Number of saccades for groups and awareness over blocks.**

Block	**EXP_CONT**	**NOEXP_CONT**	**EXP_FAMS**	**NOEXP_FAMS**	**NOEXP_AMP**
$Random$	1.84 $\pm$ 0.89	1.84 $\pm$ 0.94	2.62 $\pm$ 1.37	2.48 $\pm$ 1.69	2.07 $\pm$ 1.26
BOI	1.66 $\pm$ 0.74	1.73 $\pm$ 0.91	1.93 $\pm$ 1.03	2.09 $\pm$ 1.16	2.56 $\pm$ 1.80
$B15$	1.57 $\pm$ 0.73	1.65 $\pm$ 0.95	1.91 $\pm$ 0.99	1.96 $\pm$ 1.23	1.33 $\pm$ 0.16

Table 5.12: **Gaze onset (ms) for groups and awareness over blocks.**

Block	**EXP_CONT**	**NOEXP_CONT**	**EXP_FAMS**	**NOEXP_FAMS**	**NOEXP_AMP**
$Random$	134.20 $\pm$ 175.25	76.58 $\pm$ 222.13	94.71 $\pm$ 228.16	105.91 $\pm$ 225.53	108.18 $\pm$ 175.06
BOI	-255.62 $\pm$ 257.51	-3.42 $\pm$ 261.46	-171.33 $\pm$ 270.50	14.52 $\pm$ 224.16	92.66 $\pm$ 242.52
$B15$	-264.11 $\pm$ 261.98	-39.74 $\pm$ 276.88	-225.07 $\pm$ 246.97	50.65 $\pm$ 222.43	134.80 $\pm$ 183.96

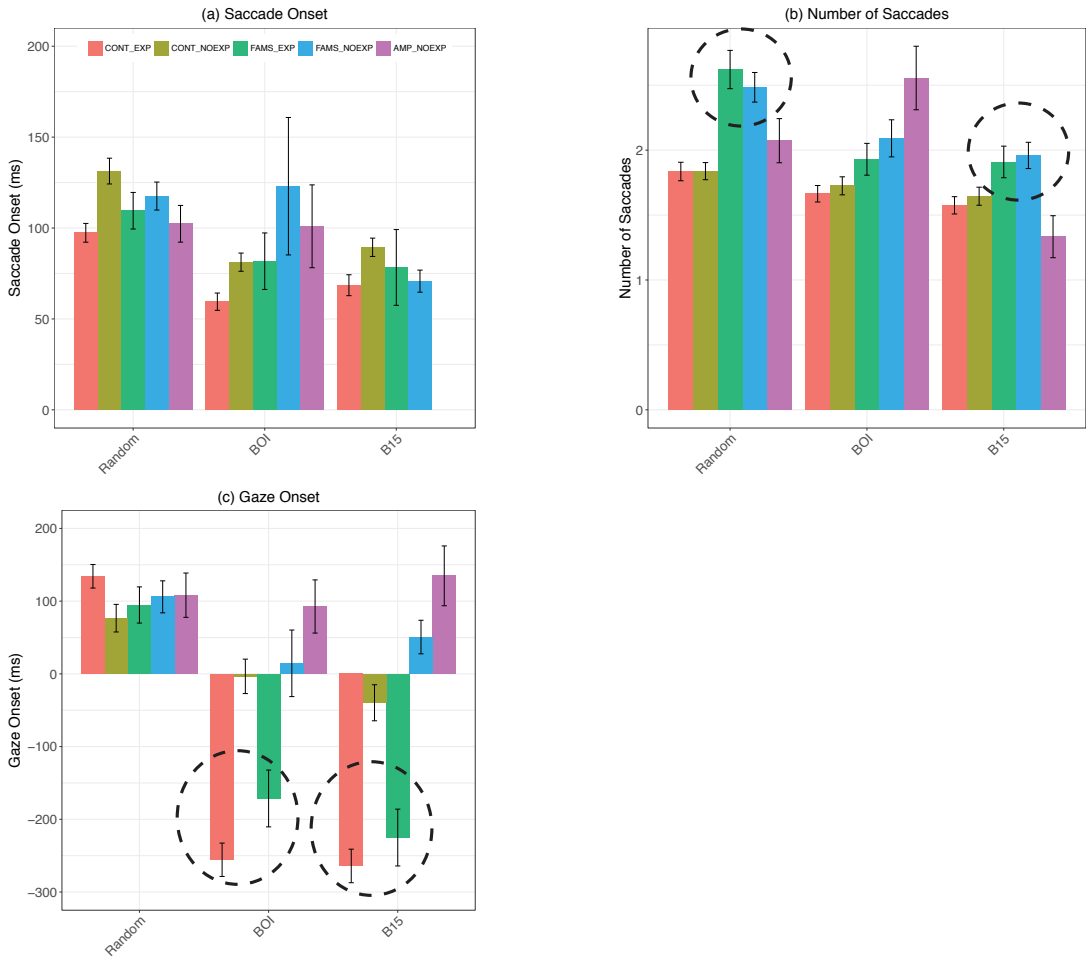


Figure 5.12: **Gaze behavior measures during Random block.** (a) Initial saccade latency. No significant difference in saccade onset latency between groups was noted at Random. All groups, with the exception of EXP_FAMS subjects, demonstrate a significant decrease in saccade onset by the end of the experiment. (b) Number of saccades per trial. FAMS subjects demonstrate significantly greater number of saccades at Random compared to CONT subjects, with a significant decrease in the number of saccades over the experiment for FAMS subjects. (c) Gaze onset latency to target of interest. Subjects demonstrated a significant decrease in gaze onset latency over the course of the experiment, with EXP subjects showing significantly more negative onset times compared to NOEXP subjects.

5.5.5 Neural correlates of kinematic and saccade behavior:

The examination of neural activity related to motor and visual behavior was conducted utilizing a sorted VEP-image approach. Electrodes of interest included those centered over the regions of interest from the visuomotor facilitative network identified in Aims 1 and 2, ACC (FZ electrode),

Table 5.13: **Gaze measures of saccades and fixation over blocks between groups and awareness.**

Model Test	Analysis	df	Saccade Onset	No. of Saccades	Gaze Onset
$M0$	Repeated measures	3			
$M0 vs. M1$	Main Effect of Group	4	p=0.51	**p=0.002**	p=0.08
$M0 vs. M2$	Main Effect of Awareness	4	p=0.25	p=0.56	**p<0.001**
$M0 vs. M3$	Main Effect of Block	5	**p<0.001**	**p<0.001**	**p<0.001**
$M1 vs. M4$	Interaction Effect of Awareness & Group	6	p=0.43	p=0.93	**p<0.001**
$M4 vs. M5$	Interaction Effect of Awareness, Group, and Block	14	**p<0.001**	**p<0.001**	**p<0.001**

SMA (CZ electrode), PCC (PZ electrode), and left and right precuneus (P3 and P4 electrodes respectively). An additional electrode of interest, F6, representing neural activity over the right prefrontal gyrus was also included. As one AMP subject was unable to finish more than 6 trials, only intact subject data was analyzed for Aim 3. Only VEP-image sorted data reflecting differences between groups are shown.

VEP images for the LPCun (Figure 5.14) and RPcun (Figure 5.16) demonstrate movement-onset related time-locked neural activity for both the EXP_CONT and EXP_FAMS subjects. EXP_CONT subjects show a strong positive deflection after movement onset, while EXP_FAMS subjects show the positive deflection ending immediately prior to movement onset . In addition, EXP_FAMS subjects demonstrate a much larger negative deflection time-locked to the stimulus onset compared to EXP_CONT subjects. For both EXP subjects, neural behavior time-locked to movement is only apparent for movement onset times greater than 180 ms. Both NOEXP_CONT and NOEXP_FAMS subjects show neural activity strongly linked with the timing of the stimulus onset, with no apparent movement-locked neural activity. The same trend is seen for all subjects over

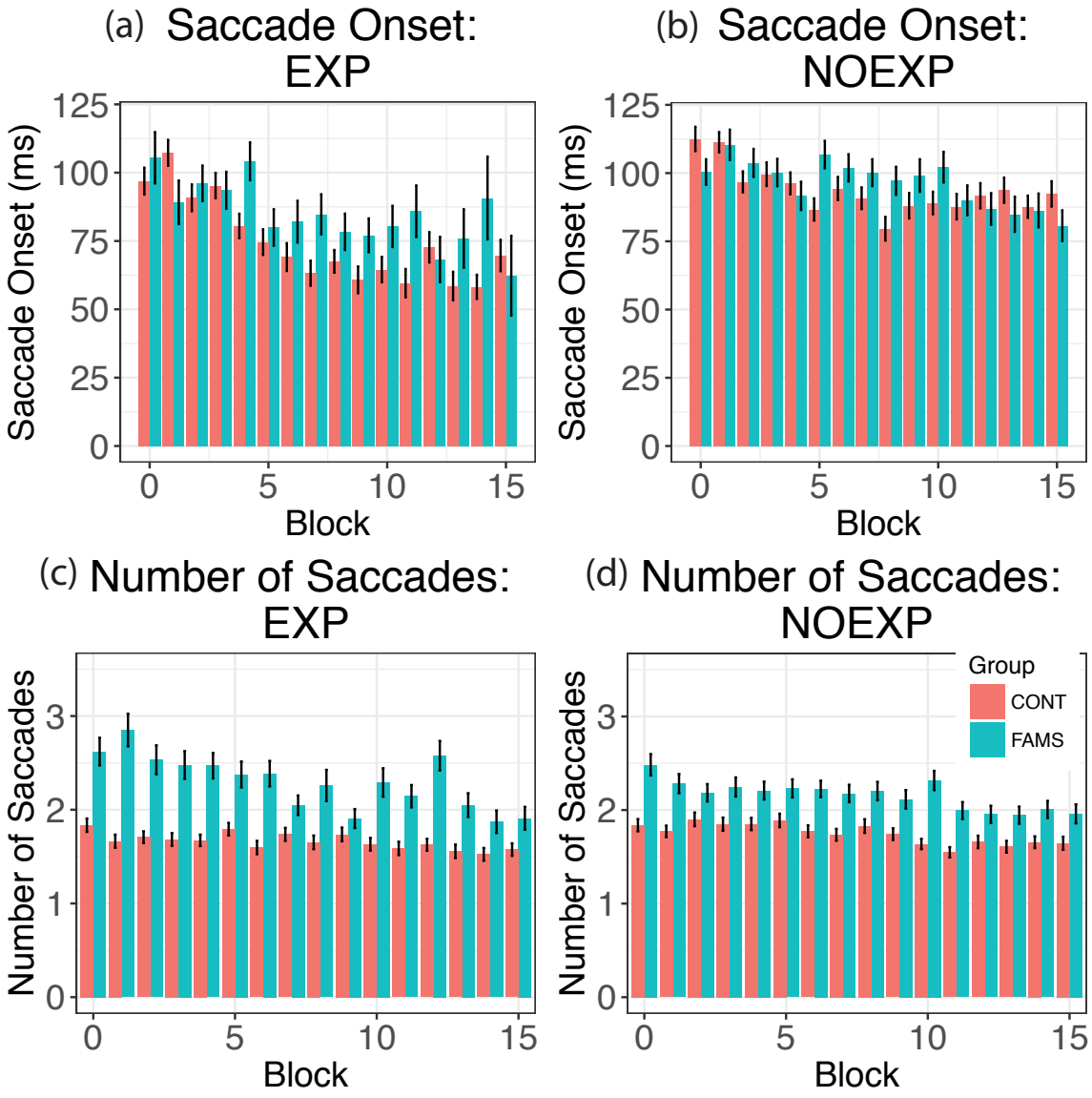


Figure 5.13: **Saccade behavior over the course of the experiment.** (a) Initial saccade latency for EXP subjects, (b) Initial saccade latency for NOEXP subjects, (c) Number of saccades for EXP subjects, and (d) Number of saccades for NOEXP subjects

the RPcun electrodes. Saccade time-locked VEP images (Figure 5.15 and Figure 5.17) show no saccade-related activity over the LPcun and RPcun for all subjects.

The SMA VEP image (Figure 5.18) for EXP_CONT shows a positive deflection around 200 ms, which is time-locked to behavior as movement onset times get faster than 300 ms, but becomes stimulus-locked once movement onset times are faster than 180 ms. EXP_FAMS subjects

demonstrate a positive deflection peaking around 200 ms, which remains time-locked to the stimulus until movement onsets are faster than 180 ms. After this point, positive deflections appear to be time-locked to movement with a delay of 100-150 ms post-movement onset. As with the precuneus regions, NOEXP subjects do not demonstrate any clear movement-locked activity. In addition, saccade-locked VEP images revealed no saccade-locked activity over the SMA electrode (Figure 5.19) .

For the rIFG electrode (Figure 5.20) , EXP_CONT subjects demonstrate multiple positive deflections, with a movement-locked deflection peaking between 200-300 ms. This activity was noted to be greatest when movement onset times ranged from 180-250 ms. EXP_FAMS subjects demonstrated a negative deflection peaking around 200 ms which showed movement-locked activity prior to movement onset. This negative deflection was most prominent for movement onset times ranging from 200-600 ms. At movement onset times faster than 200 ms, the negative deflection is minimal and a positive deflection begins to appear post-movement onset. NOEXP_CONT subjects demonstrate a sharp negative deflection time-locked to behavior appearing after 300 ms. NOEXP_FAMS subjects do not demonstrate any clear movement-locked activity over the rIFG electrode. Saccade-locked VEP images reveal no saccade-locked activity for EXP_CONT, NOEXP_CONT, or NOEXP_FAMS subjects (Figure 5.21) . EXP_CONT subjects, however, demonstrate a negative deflection time-locked to saccades at saccade latencies greater than 200 ms. When saccade latencies drop below 200 ms, the negative deflection becomes stimulus-locked in its behavior.

5.6 Discussion

The overarching goal of Aim 3 was to provide a more precise examination into the visual and motor components contributing to the incidental development of explicit awareness during a three-dimensional reach-and-grasp task. As previous studies [12] have demonstrated impaired motor learning for prosthesis users, neurobehavioral changes for subjects using a thumb and finger (CONT) to grasp the disc, as well as intact and amputee subjects using a prosthesis (FAMS and

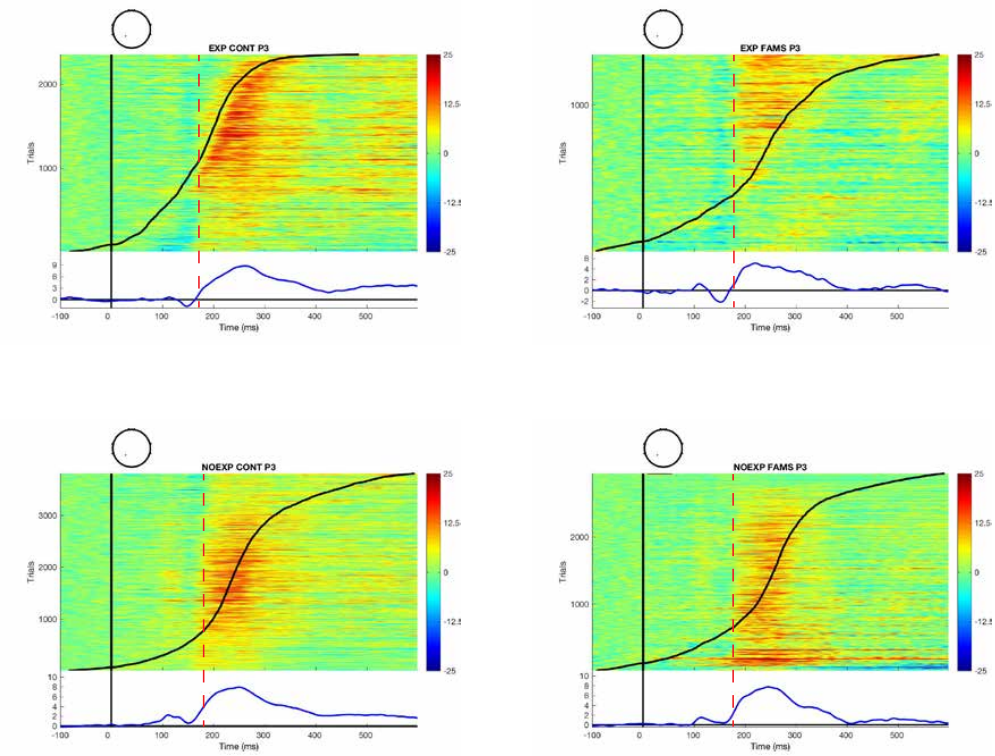


Figure 5.14: **Movement-locked VEP Image: Left Precuneus (P3 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

AMP), were examined. Subjects were classified as EXP or NOEXP based on the level of recall at the end of the experiment, with timing of anticipatory movement onset being used for identification of the behavioral block of interest for correlations with neural behavior. Five of the twelve CONT subjects (42%) and four of the twelve FAMS subjects (33%) demonstrated explicit recall, while neither of the AMP subjects demonstrated explicit recall. While these results demonstrate that FAMS subjects were able to develop explicit awareness, which differs from prior results in our lab [12], the paradigm in Aim 3 had subjects experience the sequence 75 times compared to the 48 repetitions of the sequence experienced in the Hughey study. Results showing the near significantly later onset of EXP behavior for EXP_FAMS subjects compared to EXP_CONT sub-

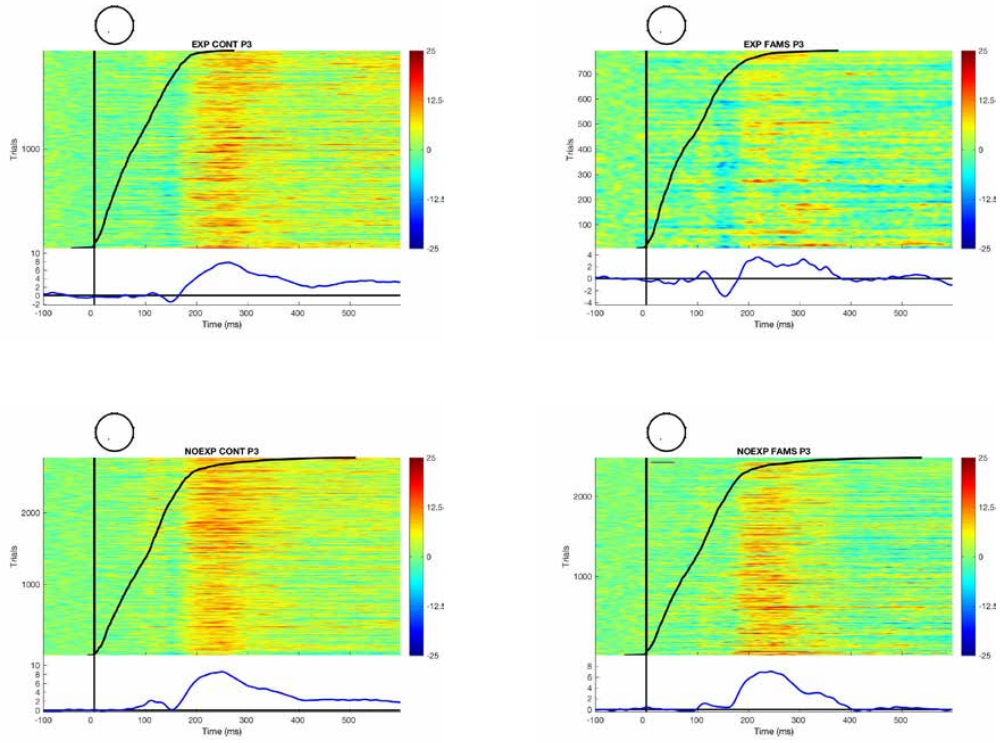


Figure 5.15: **Saccade-locked VEP Image: Left Precuneus (P3 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

jects, along with the significantly decreased level of recall for NOEXP_FAMS and AMP subjects compared to NOEXP_CONT, supports the claim that prosthesis users demonstrate impaired motor learning. The subsequent neurobehavioral analyses conducted provided insight into the role of enhanced visual reliance by FAMS and AMP subjects compared to CONT subjects in impairing motor learning processes, and the role of enhanced sensorimotor information for facilitating sequence discovery in both CONT and FAMS subjects. The results provided in Aim 3 have potential implications for rehabilitative approaches and prosthetic design to assist amputees in successfully adapting to their device.

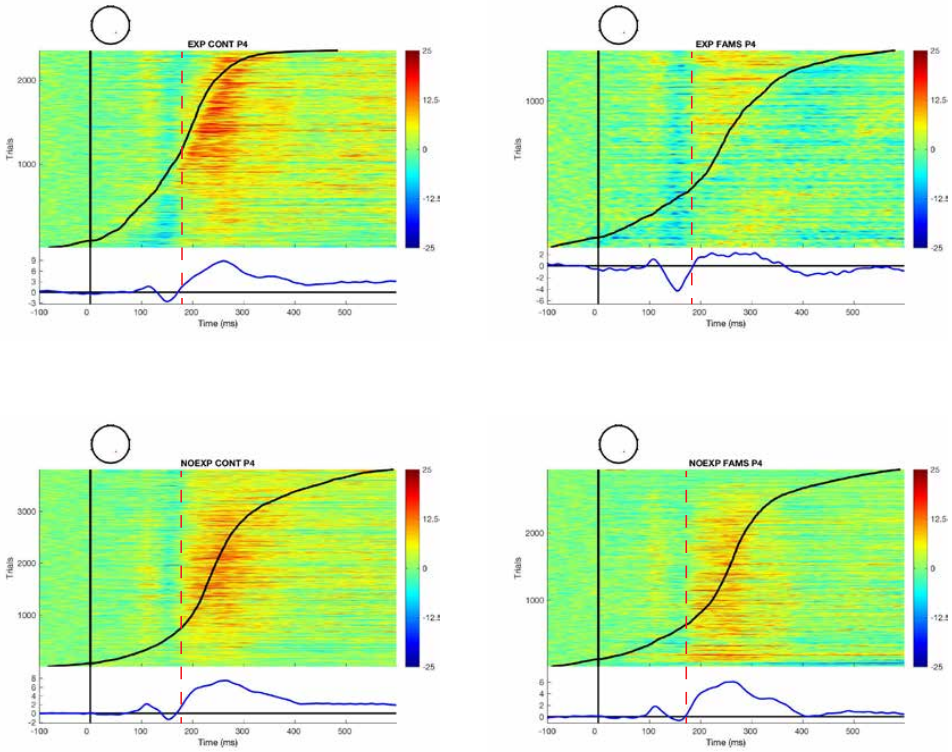


Figure 5.16: **Movement-locked VEP Image: Right Precuneus (P4 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

5.6.1 Neurobehavioral correlations with regions of the visuomotor integration network identified in Aims 1 and 2

The utilization of movement onset as an indicator of EXP behavior provided a reliability in accurately classifying awareness levels with a sensitivity of 72.7% and a specificity of 92.8%. The differences in reliability of the threshold compared to Aims 1 and 2 may be reflective of the unique differences for the reach-and-grasp task. Utilization of additional measures for awareness such as recognition of sequence and non-sequence patterns have also been utilized and may be a valuable addition for the post-experiment interview in future studies to more accurately assess the level of sequence awareness. Utilization of the timing predicted by explicit movement onset behavior

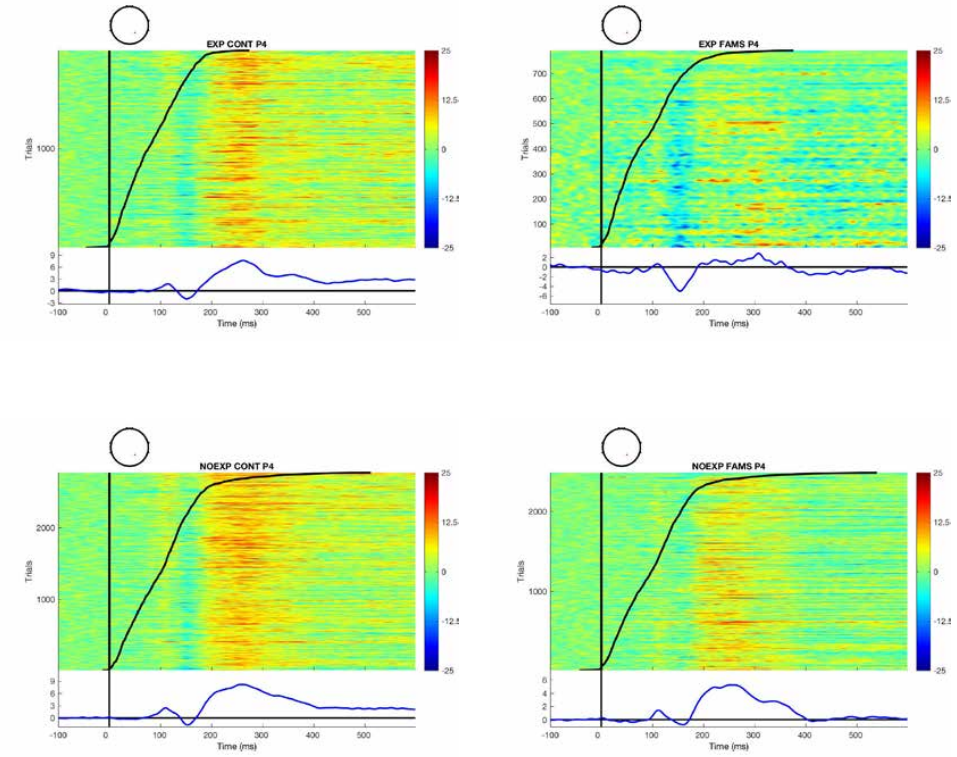


Figure 5.17: **Saccade-locked VEP Image: Right Precuneus (P4 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

revealed similar correlations with peak neural activity over the PCC and the SMA, but did not reveal significant correlations with the LPcun or ACC regions. These results provide additional evidence for the pivotal role of the PCC in recognizing the initial pattern and shifting attention to a learning-based strategy. As the SMA is attributed to internally-generated movement execution, the significant correlation for EXP behavior with the timing of SMA activity, provides evidence for the accuracy of the movement onset classifier in identifying both the presence, and timing, of explicit awareness development. The lack of correlation with the LPcun and ACC regions suggests that the reach-and-grasp task utilized the visuomotor integration process differently than in the finger-pressing task. This can be attributed to the additional resources needed to develop the

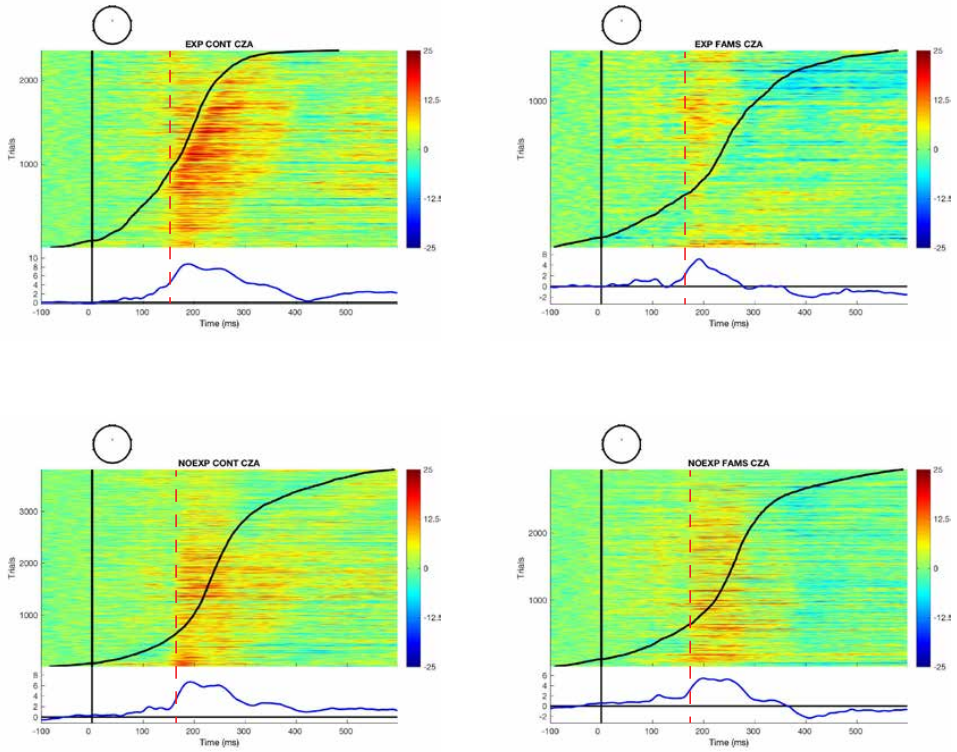


Figure 5.18: **Movement-locked VEP Image: SMA (CZA Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

more complex motor plan necessary for reach-and-grasp. While the LPcun and ACC are proposed to still be critical to sequence learning, the inclusion of online sensory feedback during reach may alter how the neural activity changes over time. This differential recruitment is additionally reflected in the lack of correlation between neural regions of the facilitative network from Aims 1 and 2.

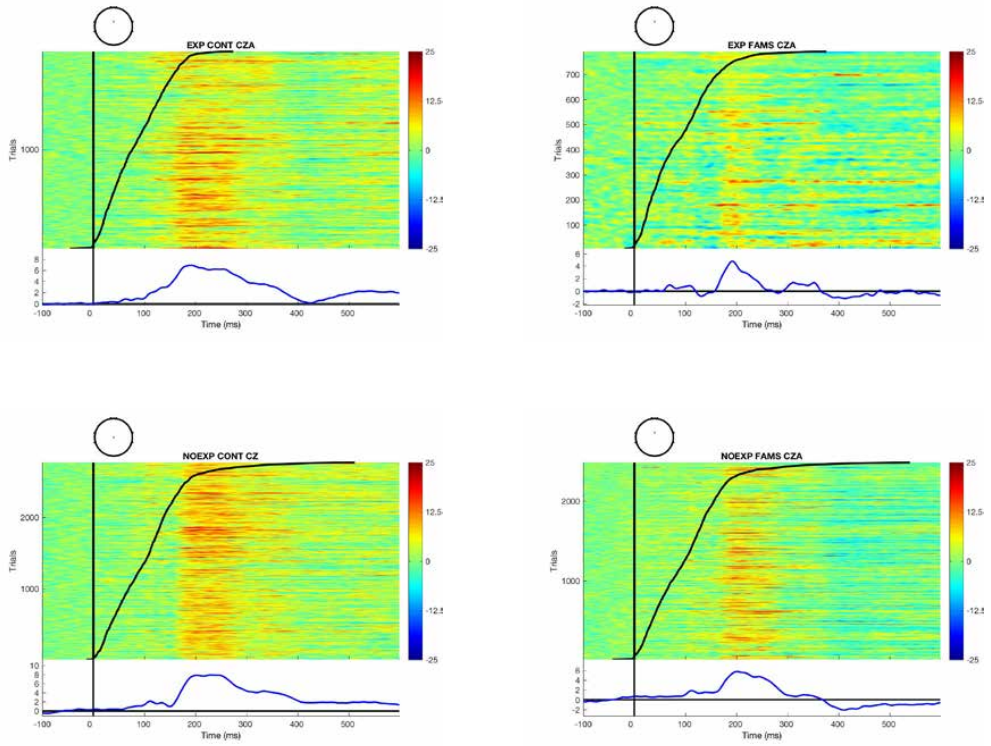


Figure 5.19: **Saccade-locked VEP Image: SMA (CZA Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

5.6.2 Behavioral correlates of explicit awareness development: The roles of working memory, visual attention and sensorimotor feedback

Results from the 2-back task supported results from Aim 2 with EXP subjects demonstrating significantly faster 2-back performance. For CONT subjects, this distinction was close to binary, with a cutoff of 1000 ms predicting all but one NOEXP subject. For FAMS subjects, however, there were four NOEXP_FAMS subjects with scores lower than 1000 ms. This wider distribution of 2-back scores for the FAMS group suggests that, functional working memory is a necessary, but not sufficient, attribute for successful motor learning when using a prosthesis.

To identify potential predictors and correlates for the development of awareness, behavioral

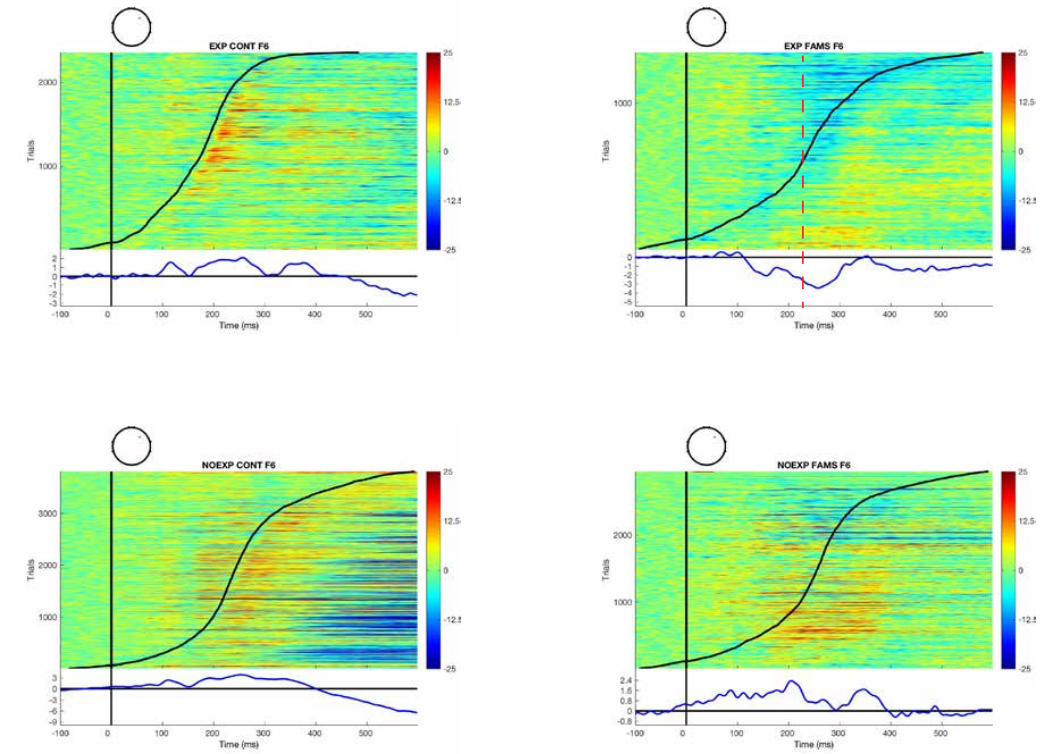


Figure 5.20: **Movement-locked VEP Image: Right IFG (F6 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

and kinematic analysis was conducted for the Random block, BOI, and B15. Kinematic measures of speed revealed EXP_FAMS subjects demonstrated a significantly faster MO at Random compared to NOEXP_FAMS subjects, suggestive of a differential strategy in responding to the stimulus from the beginning. Over the experiment, all subjects showed a decrease in MO reflective of the anticipatory behavior previously noted to be present with implicit learning [17]. Movement time measures revealed a decrease for all subjects over the course of the experiment, with EXP_FAMS subjects reaching a MT similar to NOEXP_CONT subjects. This significant change in MT may be reflective of the movement vigor associated with explicit awareness [31]. RT measures revealed EXP_FAMS subjects obtaining a significantly faster RT compared to NOEXP_CONT subjects.

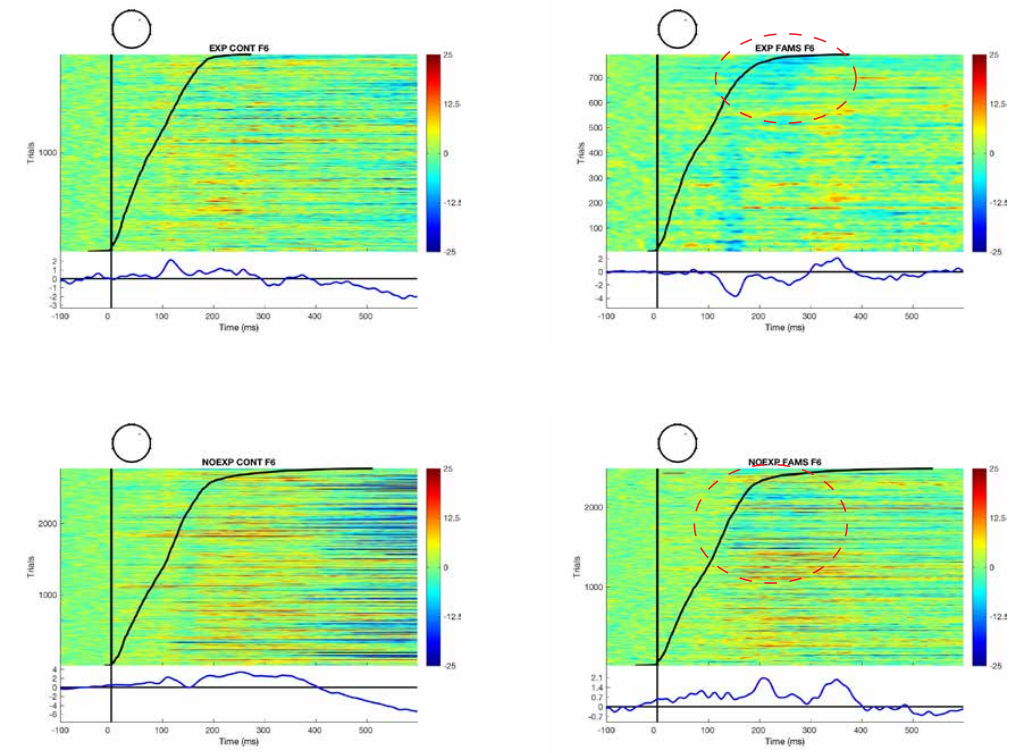


Figure 5.21: **Saccade-locked VEP Image: Right IFG (F6 Electrode).** (a) EXP_CONT subjects, (b) EXP_FAMS subjects, (c) NOEXP_CONT subjects, and (d) NOEXP_FAMS subjects

Due to the ability to separate MO and MT parameters, it was possible to attribute this significant decrease to changes in both MO and MT. While previous studies have demonstrated a decrease in MO to be associated with a concomitant increase in MT for implicit learning, the impact of explicit awareness was not explored [17]. The demonstrated decrease in MT with EXP subjects suggests subjects not only participated in anticipatory movements, but also improved movement execution times. Peak velocity changes revealed EXP_CONT subjects to have a significantly greater peak velocity than all other groups at Random. This suggests that EXP_CONT subjects were highly focused on speed for the task from the beginning. By B15, EXP_FAMS subjects demonstrated a peak velocity similar to EXP_CONT subjects. This increase in peak velocity may be reflective of

the movement vigor described earlier, as well as enhanced motor control abilities [31]. AMP subjects, while not included in statistical analyses, demonstrated behaviors similar to NOEXP_FAMS subjects for all speed-related kinematic variables.

It is proposed that the above mentioned changes in speed measures may be representative of the speed-accuracy-tradeoff [271]. Accuracy measures of directional error and lateral trunk movement provide evidence as to the accuracy tradeoff may be occurring for EXP subjects. It is proposed that the decrease in accuracy may provide beneficial, sequence-specific, sensorimotor information beneficial for acquisition of the motor sequence by EXP subjects. For directional error, EXP_CONT subjects demonstrated significantly larger directional error compared to NOEXP_CONT subjects. This increase in initial reach error may be capitalized on by these subjects to provide additional sensorimotor information regarding regularities in reach trajectories. The enhanced lateral trunk movement seen by EXP_FAMS subjects may be the result of an initial compensatory mechanism with which to accommodate the lack of supination and pronation. The larger sensory information provided by the extra movement, may result in additional sensorimotor information which facilitates the identification of a regularity in trunk movement enhancing sequence identification. From a motor control perspective, analysis of velocity variability revealed that both EXP_CONT and EXP_FAMS performed more smoothly than their NOEXP counterparts, NOEXP_CONT and NOEXP_FAMS subjects respectively. This suggests that EXP subjects may have entered the experiment with better motor control compared to NOEXP intact subjects. Velocity variability for the AMP, however, was similar to EXP_CONT subjects, demonstrating their motor control familiarity with their prosthetic device.

Gaze behavior revealed variations between groups which provide evidence for an enhanced reliance on visual feedback for FAMS subjects. Gaze behavior analysis revealed no significant differences in saccade or gaze latencies between the groups at Random supporting the assumed requirement of no visual processing deficits. There was, however, a significant difference for the number of saccades between FAMS subjects compared to CONT subjects, regardless of awareness. The number of saccades per trial demonstrated a significant decrease over the experiment

for FAMS users, potentially reflective of a practice effect with the device. A similar trend was noted for the AMP subjects. This result suggests that prosthesis users are participating in more visually-guided online control during reach-and-grasp compared to CONT subjects. This is in line with other studies that have shown an increased reliance on vision when somatosensory information is unreliable [276, 273, 275]. The enhanced reliance on vision for motor planning and online control of both the reach and grasp phase would introduce additional demands on the the visual working memory system, explaining the necessary, but not sufficient results of the 2-back score noted earlier.

5.6.3 VEP image sorting: The role of error-based learning and enhanced visual reliance

Results from the VEP-image sorting to movement onset, provided evidence that NOEXP_CONT and NOEXP_FAMS subjects remained in a stimulus-response mode of behavior throughout the experiment, in spite of demonstrating reduced movement onset latencies. EXP subjects, however, demonstrated clear movement-locked neural activity over LPcun, RPCun, SMA, and rIFG areas. This movement-locked activity provides neural evidence for a shift away from the stimulus-response mode to one which facilitates learning of the motor sequence. While both EXP_CONT and EXP_FAMS demonstrated neural activity time-locked to movement onset, the timing and deflections are unique to each group suggesting a difference in how the task and motor sequence was learned. Additionally, EXP_FAMS subjects demonstrated saccade-locked behavior over the rIFG electrode. As the stimulus-locked behavior was present for NOEXP subjects over all electrodes, the remainder of the discussion will focus on the significance of the differences in movement- and saccade-locked VEP images for EXP subjects.

For EXP_CONT subjects, movement-locked VEP image data over the LPcun, RPcun and SMA electrodes revealed a positive deflection peaking between 200-300 ms. While, when time-locked to a stimulus, this activity would be reflective of the P3, which has been suggested to reflect context updating in learning paradigms [198], in a movement-locked paradigm, this timing is reflective of a post-error positivity (Pe) [299, 73]. The Pe is a positive deflection that has been shown to be mod-

ulated by error detection and error monitoring [300, 235, 280, 277]. The movement-locked neural activity over LPcun, associated with motor planning [118], and RPcun, associated with visuomotor transformations [114, 115], for EXP_CONT subjects provides evidence for an effortful focus on context updating, via error monitoring, concerning both the spatial information regarding stimulus location and the motor plan necessary to achieve the reach goal. This movement-locked positivity is present only for movement onset times greater than 180 ms, suggesting that subjects were no longer relying on the visuospatial information for movement onset. This shift may provide a neural marker for the onset of explicit awareness. For the SMA, movement-locked activity demonstrated a similar trend for movement onset as seen with the LPcun and RPcun. For movement onset times faster than 180 ms, however, the positive deflection remained, but the peak activity demonstrated a stimulus-locked behavior. This may be reflective of the P2 VEP component noted in Aims 1 and 2 which is proposed to be associated with the internally generated execution of the movement. The consistent timing of this activity suggests that EXP_CONT subjects had not only learned the motor sequence, but the pacing of the activity as well.

EXP_FAMS subjects demonstrated a stimulus-locked negative deflection peaking at 150 ms, timing reflective of the LateN1 discussed in Aims 1 and 2. The amplitude of this activity was much greater than that seen with the EXP_CONT subjects. As described in Aims 1 and 2, the lateN1 is reflective of perceptual processing, supporting other research suggesting that using a prosthesis requires enhanced visual demands [159, 12]. The positive deflection following the lateN1, the P2, has a stimulus-locked onset, but a movement-locked offset. This difference in movement-locked activity compared to EXP_CONT subjectgs, provides evidence that EXP_FAMS subjects are utilizing an alternative neural network for error-monitoring. Activity noted over the rIFG provides insight into the learning method utilized by EXP_FAMS subjects. The rIFG has been suggested to be pivotal for inhibition, error monitoring and motor control [301, 302]. The strong negativity pre-movement onset seen for EXP_FAMS subjects over this region provides evidence that FAMS subjects developing awareness may be exhibiting an enhanced focus on error monitoring while learning to control the device at the beginning of the experiment. This focus may have provided

an opportunity to utilize the sensorimotor information regarding movement direction for the probabilistic learning of the sequence. Saccade-locked behavior over this region provides evidence for visual resources being utilized to assist in controlling movement of the device as well.

5.7 Conclusions

The results of Aim 3, utilizing a multimodal approach, provide unique insights into the neurobehavioral processes involved in motor sequence learning for both intact and prosthesis users. As previous studies have demonstrated impaired sequential motor learning for amputees [12, 159], understanding the neurobehavioral contributions behind this impairment may allow for improved rehabilitative and prosthesis design approaches. The use of a three-dimensional workspace allowed for the decoupling of visual and motor components, utilizing eye-tracking and motion detection, which could then be correlated with neural activity. Sensitivity/specificity measures, along with significant SMA correlations, provided evidence for utilizing a threshold of movement onset times faster than 110 ms for consecutive sequence repetitions a reliable indicator for both the presence, and timing, of explicit behavior. Having such a behavior-focused measure which reliably classifies awareness could be a valuable tool within rehabilitative settings.

To assist in individualizing rehabilitative paradigms, it would be valuable to have predictors regarding most efficacious approach for each patient. This approach relies on neurobehavioral predictors which appear early, allowing for an online adjustment in the best rehabilitative approach. As was noted in Aim 2, one such predictor is the patient's working memory from the start. In Aim 3, it was noted that the presence of a functional working memory was a necessary component for sequential learning with intact subjects, but does not appear to be sufficient for awareness acquisition with prosthesis users. Eye-tracking neurobehavioral measures provided evidence that this may be related to the enhanced visuospatial demands of using a prosthesis. This suggests that prosthesis users may benefit from exposure to shorter sequences when first learning motor sequence patterns.

Additionally, kinematic measures introduced potential predictors for learning the motor se-

quence which appeared as early as the first block. EXP subjects appeared to engage in a focus on speed over accuracy which may have introduced errors in behavior that were beneficial to detecting the presence, and may be necessary for strategy-switching to a learning focus. For prosthesis users, this focus on speed was noted to be associated with increased lateral trunk movement. While the enhanced sensorimotor information provided may be beneficial in acquiring the sequence, it is unclear if this compensatory behavior would introduce a risk for overuse injuries.

The time-locked VEP images provided additional neurobehavioral evidence for the pivotal role of error-based learning in both intact and FAMS subjects. While both groups were able to acquire the sequence, prosthesis users took longer for explicit behavior to appear. This provides evidence that learning to control the device is required before the regularity of target locations can be attended to for sequence identification. As it is proposed that the delay in learning the sequence is due to the visual working memory being utilized for controlling the device, it would be valuable to examine the utility of introducing additional, sequence-related sensorimotor information for prosthesis users. This additional information may provide an opportunity for probabilistic learning while allowing the visual system to focus on providing the online feedback information necessary for a successful reach.

CHAPTER 6

INTEGRATION

6.1 Summary

The overall goal of the dissertation was to examine the neurobehavioral changes associated with the development of explicit awareness during the learning of a sequential motor task from an individualized perspective. Sequential motor tasks are ubiquitous in daily activities and require the ability to develop the skills to execute such tasks in a fluid, automatic manner. Learning such sequential motor tasks require a complex interaction between multiple brain regions, with the rate at which skill acquisition occurs depending on the individual. This individual difference is most apparent for certain populations and disease states which have demonstrated impaired sequential motor learning [2, 6, 8, 10, 12]. Understanding the neurobehavioral changes associated with motor skill learning in healthy individuals may provide insight into potential intervention methods which may improve the motor skill learning of such populations. Motor learning has been noted to occur in stages, including both a fast and slow learning phase [36, 37]. The current study focused on neurobehavioral changes associated with the fast, within session, motor learning phase. Many previous studies examining motor learning have done so from a group analysis approach, potentially masking transient neurobehavioral changes associated with the learning process [92]. Analyzing neural changes from an individualized perspective would have the potential to identify such transient neurobehavioral changes. To address the goal of this thesis, a neurobehavioral indicator of motor learning was utilized to identify neurobehavioral changes correlative with motor sequence learning which are reflective, or predictive, of the learning progression with a motor sequence. The common experimental paradigm of an SRT task was utilized to examine motor learning, with incidental development of awareness as a marker for an individual's learning rate. Results from Aim 1 provided a reliable, indirect, behavioral indicator for the presence, and most importantly, the

timing, of incidentally developed explicit awareness during the SRT task. The timing of explicit behavior was found to be correlative with neural activity over frontoparietal regions previously shown to be involved with visuomotor integration processes [98, 94]. Neural correlations revealed the presence of a facilitative frontoparietal visuomotor integration network for subjects developing explicit awareness. Results from both Aim 1 and Aim 2 suggests a pivotal role of the PCC in identifying a regularity in the environment, upon which a strategy shift occurs, moving away from a stimulus-response behavior, to one which incorporates a probabilistic learning behavior for explicitly aware subjects [228]. Subjects failing to develop awareness demonstrated neural activity which suggested they maintained a stimulus-response behavior throughout the experiment. Results from Aim 2 provided additional evidence for the importance of working memory in sequence acquisition. For subjects demonstrating explicit behavior of the priming sequence, results also suggested that the strategy used for learning the initial sequence impacts the ability to generalize to a novel, more complex task within session. Aim 3 results provided insight into the impaired motor sequence learning observed for prosthesis users [12, 159]. Prosthesis users demonstrated saccadic and neural behavior reflective of the increased visuospatial demands required for controlling the prosthesis which altered the ability to identify the environmental regularities of the presented sequence. Results also suggested that behavior which provided additional sequence-specific sensorimotor information may facilitate the learning of a sequence for prosthesis users. Key points with respect to the individualized approach and the proposed facilitative frontoparietal network will be expanded upon below with reference to the roles of working memory, strategy selection and the provision of additional sequence-specific sensorimotor information in sequential motor skill acquisition.

6.2 Detecting both the presence of, and timing of, incidentally developed awareness

Examination of the neurobehavioral changes associated with sequential motor learning from an individualized perspective relies on the ability to know not only if, but when, learning has occurred. Many studies have utilized the presence of explicit awareness as a marker reflective of learning [64,

54, 17, 25], but have done so utilizing an intentional paradigm in which subjects were informed of the presence of a sequence. This approach allows for direct assessment of awareness throughout the experiment, but may recruit explicit system neural resources differentially from that seen with the incidental discovery of a sequence. As described in Chapter 1, the identification of the presence, and timing, of such incidentally developed awareness required a more indirect measure of awareness. The presence of explicit awareness would provide an individual the opportunity to execute the appropriate movement prior to the corresponding stimulus. Behaviorally, this was seen as movement times which were significantly faster than those noted at baseline. Neurally, the internally generated execution of the response would be seen as enhanced activity over the SMA. Results from Experiment 1 of Aim 1 provided an indirect, individualized threshold indicator for the presence of incidentally developed explicit awareness based on reaction time latency changes relative to baseline performance. Behavioral validation of the indicator in Experiment 2 of Aims 1 and 2 demonstrated a reliability measure of over 90% in both sensitivity and specificity for accurately classifying subjects as explicitly aware. Neural correlations between the timing of explicit behavior and the timing of peak SMA activity revealed a significant correlation. This evidence provided neural validation that the indicator was successful in identifying both the presence, and timing, of the presence of explicit awareness. The use of reaction time as the behavioral measure in Aims 1 and 2 did present the potential confound of not separating the contributions between movement onset and movement execution. Aim 3 took advantage of an experimental paradigm which allowed for a clear distinction between movement onset and movement times. For Aim 3, utilization of movement onset times determined to be anticipatory (faster than 110 ms), provided a reliable behavioral indicator for the presence of explicit behavior. As in Aims 1 and 2, the neurobehavioral correlations for the timing of explicit behavior with peak SMA activity provided neural validation for the movement onset indicator identifying both if, and when, awareness occurred in a three-dimensional paradigm.

6.3 Facilitative frontoparietal network

Having an indicator for the presence, and timing, of explicit awareness provided the opportunity to establish an individualized learning rate with which to examine neural changes. Results from Aims 1 and 2 revealed the presence of a facilitative frontoparietal network associated with explicitly aware subjects, which was subsequently validated in Aim 2 (Figure 3.9). The proposed facilitative network was identified by examining neural regions demonstrating significant correlations for peak activity timing with the timing of explicit behavior. In ERP studies, deflections are seen in both a positive and negative direction. The variation in direction of the deflections are influenced by factors such as the type of neurotransmitter involved, the orientation of the neural generators with respect to the scalp electrode, and the location of the reference electrode [178]. Therefore, directionality of the ERP is of less importance than the amplitude of the deflection. The amplitude of an ERP deflections are proposed to be reflective of the level of neural activation in the cortical areas underlying the electrodes [184, 178]. Therefore, it was expected that the timing of peak ERP area amplitude would indicate peak neural activation for the neural regions examined.

The ERP components of interest were the EN1, LN1, P2 and P3. Functionally, the amplitudes of these ERP components have been shown to be sensitive to specific visual processing and higher level cognitive activities. The EN1 is typically seen with a more frontal spatial orientation and is attributed to attentional gating neural processes [185, 138]. The LN1 has a more posterior distribution and is associated with visual processing activities [187, 189]. The P2 typically demonstrates a centroparietal localization and has been shown to be involved with sensorimotor comparisons and is noted to be enhanced with conscious awareness [193, 194, 185, 195, 196]. The P3 is a complex component attributed to multiple processes, most notably being modulated by novelty and involved with context updating in learning paradigms [196, 198, 199]. Aim 1 dipole examination provided estimates for localization of the neural generators for the aforementioned ERP components. The EN1 component was attributed to the ACC, the LN1 to the LPcun and RPcun, the P2 component to the SMA, and the P3 component to the PCC. The results are in line with other studies examining

the neural generators of the ERP components examined [188, 192, 194, 195, 196].

From a functional perspective, the regions described above have all been shown to play a role in visuomotor tasks. The ACC is attributed to multiple neural processes including attentional gating, error monitoring, reinforcement learning and motivation [108, 107]. Both the LPcun and RPcun are known to be involved in visual processing with a noted hemispheric specialization. The RPcun has been shown to be involved in processes requiring visuospatial transformations [116, 115, 117], while the LPcun is attributed to visuomotor planning [303, 118, 120]. As mentioned previously, the SMA has been shown to be reflective of movements which are internally-executed [133, 55, 130, 131]. The PCC is been noted to be an area involved in the identification of environmental regularities and inducing a shift to behavior more optimal for achieving the current goal [111, 112, 113].

While the timing of peak ERP activity over the regions described above all demonstrated neurobehavioral correlations with the timing of explicit behavior, it was also noted that four of these regions demonstrated linear correlations with each other. Peak activity occurred first over the PCC, followed by the LPcun, with the SMA and ACC demonstrating peak activity at similar times. This linear correlation was not present for subjects not demonstrating explicit behavior, suggesting activation of the observed facilitative visuomotor integration network was necessary for development of explicit awareness. Based on the above mentioned functions associated with the ERP components and neural regions examined, it is suggested that the PCC is pivotal for activation of the facilitative network. The PCC, as mentioned above, is involved in identification of regularities which may be able to be capitalized upon by a behavioral strategy shift. The strategy shift proposed is one from a stimulus-response behavior directed by the RPcun, to one of a learning strategy facilitated by the visuomotor planning capabilities of the LPcun. The visuospatial information provided by the RPcun and the predicted upcoming visuomotor plan from the LPcun provides sensorimotor information for comparison by SMA regions, with compatibility information providing the ACC with information necessary for probabilistic learning.

6.4 Role of working memory capacity in motor sequence learning

Results of Aim 2 and Aim 3 introduced information regarding the role of working memory capacity for the development of explicit awareness in sequential motor learning. Multiple studies have shown the importance of working memory capacity in the acquisition of motor skills [152, 153, 154, 156, 157]. From the perspective of the facilitative network, successful activation of the PCC relies on the ability to recognize the regularity present within the presented sequence. The identification of a regularity is proposed to involve a probabilistic, Hebbian-like learning process which incorporates incoming sensory information and compares it with similar information recently experienced [140, 240, 213]. For the current study, the primary sensory information provided was the visual cue regarding the current target location. Therefore, recognition of the repetitive nature would rely not only on functioning predictive learning processes, but also be influenced by the individual's visuospatial working memory capacity. The potential role of functional working memory was noted in results from both Aims 2 and 3, with individuals demonstrating slower performance on the 2-back working memory assessment failing to develop explicit awareness. Additionally, neural data from all three Aims revealed that these subjects remained in a stimulus-response behavior throughout the experiment, indicating the lack of a strategy shift by the PCC. Aim 3 also provided evidence that visuospatial working memory was primarily utilized for discovery of the motor sequence presented. Prosthesis users provided behavioral evidence that, while a functioning visuospatial working memory is necessary, it is not sufficient for sequence identification. As the presence of a prosthesis was the primary variable for the healthy subjects tested in Aim 3, this provides evidence that an additional barrier is introduced by the use of a prosthetic, similar to results previously seen in our lab [12]. The proposed source, and subsequent effect, of this barrier is discussed in more detail below.

6.5 Role of learning strategy in generalization

Results from Aim 2 provided evidence implicating the role of learning strategy in the ability to generalize sequential learning to a novel, more complex task within a single session. While learning a specific motor sequence is valuable in daily activities, it is also important to be able to transfer the acquired skills to similar, but different scenarios. Previous studies have demonstrated explicit awareness to be detrimental on generalization within session, and is proposed to rely on the consolidative processes provided by sleep [23, 52, 80]. Subjects demonstrating explicit behavior on the priming sequence fell into two subsequent groups upon transfer, those who developed awareness (EXP) and those who failed to develop awareness (EXP_NOEXP) of the novel, more complex sequence. Utilization of the individualized indicator, again, provided an opportunity to examine neural changes over the course of learning based on each individual's learning rate. This individualized analysis revealed unique activation patterns between the two groups suggestive of different learning strategies utilized during acquisition of the priming sequence. Studies of probabilistic learning have proposed two strategies available for pattern learning: (1) a memorization strategy which matches the exact statistical order of stimuli, and (2) a maximization strategy which focuses on the probabilistic relationship between successive stimuli [256, 253, 304]. A recent study by Giorgio et al. provided evidence for distinct neural networks being utilized for the two learning strategies [256]. The memorization strategy was noted to primarily recruit precuneus regions, while the maximization strategy demonstrated activation of a cingulo-striatal network. EXP_NOEXP subjects demonstrated neural activation indicating reliance on the precuneus-driven memorization strategy, while EXP subjects demonstrated neural activity reflective of cingulate-driven maximization strategy. As the maximization strategy may involve the additional recruitment of striatal circuitry, this method is proposed to facilitate consolidative processes, decreasing the risk for interference effects upon transfer [23, 49, 305, 306]. EXP_NOEXP subjects utilizing the memorization strategy establish a more fragile memory trace of the sequence which is susceptible to interference, requiring additional time for the necessary consolidative processes to occur. As both EXP and EXP_NOEXP

subjects demonstrated neural activation changes over precuneus regions, it is suggested that both groups begin with a precuneus-driven strategy, but EXP subjects move to a maximization strategy. The observed release of the PCC for the EXP subjects, but not the EXP_NOEXP subjects, is suggested to be a necessary step for this strategy shift to occur. What remained unclear from results in Aim 2 was what facilitated this PCC release for EXP subjects. Results from Aim 3 providing insights into this question are discussed later.

6.6 Motor sequential learning with a prosthesis

Aim 3 introduced neurobehavioral information regarding differences in sequential motor learning for prosthesis users compared to healthy, intact control subjects. Previous research has demonstrated impaired sequential motor learning for prosthesis users and has been proposed to be related to the enhanced visual reliance for controlling the prosthetic device [159, 307]. The paradigm in Aim 3 required a reach-and-grasp process for successful task completion. This paradigm shift, along with the inclusion of eye-tracking measures, provided the opportunity to examine, separately, the neurobehavioral correlates associated with kinematic and gaze patterns during sequential motor learning. A reach-and-grasp task has been proposed to involve motor plans unique to both the reach and grasp phase [274, 289]. The reach phase requires a motor plan incorporating the visuospatial information regarding the target location and the sensorimotor information regarding the current location of the limb being used to reach to the target location [290, 273]. Both the target and limb location information is readily accessible for both control and prosthesis users. The motor plan required to execute the reach phase, however, will be different for the two groups due to the altered inertial properties introduced by the prosthetic device for FAMS subjects and the lack of distal proprioceptive information available for amputees. The grasp phase requires a motor plan which places the hand, or terminal device, in a position to grasp the target object, as well as sensorimotor information to inform the individual when grasp has been successfully obtained [308, 309, 300]. For healthy, intact individuals, this information is provided both visually and from tactile feedback of the fingers. For prosthesis users, however, the tactile information is not available,

therefore increasing reliance on visual feedback for identification of a successful grasp.

Behavioral results examined for the first block demonstrated the impact of adjusting to the inertial properties of the prosthesis for FAMS users as seen by the significantly slower reaction times and increased movement variability noted for FAMS users. Eye-tracking measures showed that, while there was no difference between groups for saccade or gaze onset latencies, FAMS and AMP subjects engaged in significantly more saccades during the reach phase. This increased number of saccades remained elevated throughout the course of the experiment. Saccades are fast eye-movements utilized to alter the foveal focus of attention [295]. The increased number of saccades for FAMS and AMP subjects suggests multiple shifts in visual focus from the target to the prosthetic to assist in online visual feedback and movement correction [273].

We hypothesized that such enhanced reliance on visual feedback would be revealed in differential activation patterns over the RPCun and LPcun visual processing regions between groups. Initial examination failed to reveal significant differences, therefore, a time-locked ERP image approach was utilized. The time-locked ERP image methodology introduces a means with which to identify trends in neural activity which are correlated with movement or saccade behavior which may be masked when time-locking to stimulus onset [296, 295]. The primary differences between groups were noted with ERP-images time-locked to movement onset. Results over precuneus regions demonstrated distinctly different neural activation patterns between EXP_CONT and EXP_FAMS subjects. EXP_CONT subjects demonstrated the onset of time-locked neural activity post movement onset, while EXP_FAMS subjects showed an offset of time-locked neural activity pre movement onset. Additionally, EXP_FAMS subjects showed a much greater LN1 deflection compared to EXP_CONT subjects. These results suggest EXP_FAMS subjects are processing the visual information differentially, with enhanced neural resources being used to process the incoming visual information, providing neural evidence for the proposed enhance reliance on visual feedback during the reach phase.

The impact of such enhanced visual reliance was noted in both the time required for sequence acquisition and the reduced predictability of working memory capacity. EXP_FAMS users took a

significantly longer time to demonstrate behavior predictive of awareness (12.5 blocks), compared to EXP_CONT subjects (9 blocks). Additionally, over half of the FAMS subjects failing to develop awareness presented with a 2-back working memory score suggestive of being able to identify and learn the sequence. It is proposed that the enhanced reliance on visual feedback for online control take priority within the visuospatial working memory of prosthesis users, limiting the ability to rely primarily on visual information for pattern identification. This implies that prosthesis users developing awareness are utilizing alternative sensorimotor information with which to identify the regularity as discussed below.

6.7 Value of additional sequence-specific sensorimotor information in motor sequence learning

Neurobehavioral results from Aim 3 provide evidence for the facilitative benefits of using sensorimotor information, in the context of error-based learning, for sequential motor learning. Behavioral results demonstrate significant performance differences between awareness groups present from the first block of exposure. Both EXP_CONT and EXP_FAMS subjects show faster reaction times and peak velocity profiles during reach. The noted increased speed was accompanied by kinematic differences suggestive of a SAT which may beneficially introduce sensorimotor information regarding directionality of each reach. The availability of such additional sequence-related information may augment the visual information provided by the stimulus. For some EXP_CONT subjects, this may provide the additional information necessary for the strategy shift noted in Aim 2, allowing them to switch to a cingulo-driven maximization strategy. EXP_FAMS subjects demonstrated significantly greater lateral trunk movement, which provides sensorimotor information regarding sequence-related directionality of each reach. The additional sensorimotor information provided by such enhanced trunk movement is proposed to facilitate pattern recognition compensating for the visuospatial resources being used for online visual feedback. The enhanced lateral trunk movement, compared to NOEXP_FAMS subjects, remained throughout the experiment, suggesting the incorporation of the movement as part of a strategy for working the prosthetic device. While this

method may facilitate sequence learning, it is unclear the detrimental effects of such compensatory behavior.

Neural support for utilization of the additional sensorimotor information is reflected in the neural activation patterns over the rIFG electrode for EXP_FAMS subjects. The rIFG has been noted to be involved in error monitoring, inhibition and motor control processes [301, 302]. Saccade-locked ERP images revealed no saccade-locked behavior with the exception of the rIFG electrode for EXP_FAMS subjects for saccade latencies slower than 200 ms. In addition, the movement-locked ERP image over the rIFG electrode revealed movement-locked neural activity for EXP_FAMS subjects. This ERP image demonstrates enhanced negativity with a stimulus-locked onset and a movement-locked offset. The saccade-locked and movement-locked ERP images over the rIFG suggest EXP_FAMS subjects are engaging error monitoring resources related to both stimulus and movement-related information.

6.8 Neurobehavioral predictors of motor sequence learning

In addition to revealing neural activation differences between explicitly aware controls and prosthesis users, the time-locked ERP images also revealed unique differences between explicit and non-explicit subjects in both groups. EXP subjects clearly demonstrated neural activation patters time-locked to movement onset suggesting neural resources focused on information related to movement patterns. This information appears to be necessary during learning of the sequence, but becomes less relevant upon discovery of the full sequence. The only neural activation pattern which remained for EXP_CONT subjects was over the SMA, which shifted to a stimulus-locked pattern. The observed stimulus-locked behavior over the SMA provides evidence that EXP_CONT subjects had learned both the pattern and pacing of the experiment. Non-explicit subjects demonstrated neural activity patterns which remained stimulus-locked throughout the experiment, regardless of movement onset times, providing additional neural evidence that NOEXP subjects remained tied to a stimulus-response mode of behavior. The presence of this neural activation pattern regardless of movement onset times introduces the possibility of using this measure as a

tool for predicting the neural state of an individual during sequential motor learning. For subjects retaining a stimulus-response mode of behavior, additional interventions as proposed below may be introduced to induce a shift to a learning behavioral strategy.

6.9 Limitations and future directions

There are many results and implications from this dissertation which warrant further investigation to provide additional insight and clarification. The utility of an individualized approach revealed the presence of a proposed facilitative frontoparietal visuomotor integration network utilized by healthy, intact participants developing explicit awareness. Aims 2 and 3 provided evidence for beneficial effects of such incidentally developed awareness in both transfer of motor skills and movement execution. The beneficial effects of awareness on transfer was shown to be dependent on learning strategy, with subjects engaging in a cingulo-driven strategy expressing greater transfer. The beneficial effects on movement execution were noted in Aim 3, with EXP_FAMS subjects demonstrating faster movement execution without sacrificing movement variability compared to NOEXP_FAMS subjects. These results provide evidence that the development of incidental explicit awareness may provide therapeutic benefits for upper limb amputees learning to use a prosthetic device. There are, however, many additional studies necessary to address the current study limitations and further identify the most efficacious type and dosing of intervention for this amputee population.

6.9.1 Amputee subject recruitment

One primary limitation that should be addressed is the limited sample size provided in Aim 3, particularly for amputee subjects. While Aim 3 provided twelve subjects using a prosthetic (FAMS), it is unclear how closely this model reflects the motor learning processes occurring for amputees. While the one AMP subject completing the entire experiment did demonstrate similar SAT gains as the NOEXP_FAMS subjects for the time variables, the trend was opposite for peak velocity, suggesting a difference in movement execution over the reach profile between the two groups.

The recruitment of additional amputee subjects will help to identify how unique, or common, this trend is for the amputee population. Additionally, as all FAMS subjects were naive to the use of the device, how much of the performance gains noted are attributed to sequential learning versus learning to use the device itself is unclear. Both amputees recruited had utilized a prosthetic device for decades, therefore the role of learning to use the device may present a significant confound. The recruitment of additional upper limb amputees will allow for comparison of the neurobehavioral correlates with sequential learning to assess the potential impact of cortical reorganization proposed to occur with amputation [159]. How this reorganization may affect the proposed facilitative network, and the subsequent acquisition of a sequential motor skill, requires additional exploration. Identifying how closely the FAMS mirrors the neurobehavioral correlates of motor learning attributes in upper limb amputees is of extreme importance as more researchers adopt this methodology to study prosthesis use with intact subjects.

6.9.2 Machine learning approach

The introduction of a three-dimensional sequential task, along with the inclusion of eye-tracking in Aim 3 produced multiple results which were not examined in the current dissertation, but deserve further analysis. The examination of kinematics for this study was restricted to the beginning and end behavior of subjects regardless of learning rate. The complexity of kinematic changes precluded the ability to conduct a thorough examination of the changes within the context of this dissertation, but it may be found to reveal valuable insights into the proposed SAT for EXP subjects. A machine learning approach with regards to changes in initial direction, movement smoothness and grasp profile could provide evidence for when, or if, errors were associated with specific neural changes. Such an approach would allow for a more precise, trial-by-trial analysis of neurobehavioral correlates associated with error-based learning, potentially elucidating which movements were most relevant for individuals when learning the sequential motor task. From a rehabilitative perspective, understanding the kinematic changes associated with improved movement execution times may provide insights into the most efficacious portion of the reach movement with which to

focus interventions to elicit the therapeutic benefits of awareness development.

6.9.3 Transfer phase of Aim 3 task

The task utilized in Aim 3 involved a reach-grasp-and-transfer process for each trial completed. As data analysis for the presented dissertation focused on the reach-and-grasp phase, it is unclear what effect explicit awareness has on the transfer phase of the task. Due to the sequential nature of the paradigm, subjects were not only reaching to locations in a sequential order, but the location at which the disc was placed was also sequential in nature. This provides an opportunity for subjects to develop a more complex anticipatory motor plan which includes both the reach and transfer aspect of the movement. Such an anticipatory motor plan could allow for improved movement execution to the empty target location. Examining parameters such as movement time, movement trajectory, and accuracy of placement would provide a measure of how explicit awareness impacted the transfer phase. A recent study by Coats et al. [310] demonstrated altered gaze behavior in older adults during the transition between the reach-and-grasp phase and transfer phase compared to young adults, with older adults showing increased dwell time during grasp. The increased dwell time is proposed to be reflective of increased visual reliance for motor control during grasp. Measuring the dwell time for subjects in Aim 3, would provide an additional measure of how explicit awareness impacts movement execution. Combining the results from Aim 3, focused on the reach-and-grasp phase, with data from the transfer phase, will provide a more complete picture of how explicit awareness impacts movement execution in a simple reach-grasp-transfer task. As the task utilized in Aim 3 is reflective of the common activity of moving an object from one place to another done multiple times a day, this more complete picture will be important for rehabilitative practices.

6.9.4 Inclusion of pre/post assessment utilizing ADL's

In addition to the recruitment of more amputees and examining available data further, future studies examining incidental explicit awareness should utilize a pre/post assessment paradigm with

activities of daily living (ADL's). The current results demonstrate performance benefits within the context of the experimental paradigm, but do not assess the generalizability to functional activities performed outside the laboratory setting. Providing a pre/post assessment conducted with a minimum of a 24 hour window would also allow for exploration into the retention of motor skills learned during the training session. This extended pre/post time period may provide additional insight into the proposed need for consolidation with the EXP_NOEXP subjects noted in Aim 2. Identifying the impact of awareness on ADL functionality will provide a powerful measurement tool with which to assess the efficacy of any sequential motor learning training for rehabilitation. The presence of a benefit for ADL functionality would demonstrate a need to further explore methods in which to elicit explicit awareness for all patients. Approaches for this are discussed below with reference to additional sequential learning methodologies, modulating functional working memory capacity and providing additional, sequence-specific, sensorimotor information.

6.9.5 Addressing fatigue issues

An additional incidental finding in Aim 3, which warrants additional exploration, was the occurrence of fatigue for one FAMS subject and one AMP subject. The mass of the prosthetic being used introduces a potentially limiting factor in what constitutes a reasonable amount of time for within session rehabilitative interventions. An important first step in addressing this major concern would be to identify if the proposed benefits of incidental explicit awareness can be elicited with a shorter sequence of 4- or 5-elements. Aim 3 also provided valuable information regarding the distinct roles of visual and sensorimotor information for acquiring sequential motor skills. Results suggest that the availability of visuomotor working memory capacity was a limiting factor for prosthesis users. Other studies, utilizing eye-tracking, have shown specific gaze behavior changes associated with both motor sequence learning, as well as observational sequence learning [76, 311, 270, 312, 87, 268, 313, 97]. Recent studies in our lab have also demonstrated the utility of observation of a video with a prosthesis user is beneficial to the acquisition of motor control with a prosthesis [175, 294]. Such observational learning could provide a method with which to augment the pace at which

motor sequential learning could be acquired. Results from Aim 3 demonstrated that prosthesis users developing awareness participated in behavior which may have provided valuable sequence-specific sensorimotor information. This additional information may facilitate motor sequential learning when using a prosthesis due to visual resources being utilized for controlling the device. Due to the lack of sensory information available from the prosthetic device, multiple studies have conducted studies exploring the most effective form with which to provide sensory information to amputees, including tactile vibration and direct sensory fiber stimulation [308, 314, 315, 159]. Examining the efficacy of providing such additional sensory information, in a sequence-relevant form may enhance the ability of amputees to incorporate this externally-delivered information into motor plans.

6.9.6 Assessing retention of motor skill

The current dissertation Aims focused on neurobehavioral changes associated with motor skill learning from a within session paradigm. While this may be reflective of changes observed during a single rehabilitative session, it does not address the long-term retention of any noted beneficial changes. It has been shown in multiple studies that motor learning occurs on both a short-term, fast phase, and long-term, slow phase, scale [36, 40]. Future studies utilizing the presented paradigm would benefit from the inclusion of multiple sessions conducted over days, weeks, and even months, to examine the impact of incidentally developed explicit awareness on the slow phase learning process. A long-term study would introduce additional information regarding the role of consolidative processes proposed to be needed for the EXP_NOEXP subjects in Aim 2.

6.9.7 Utilizing sequential learning to improve force modulation

Information provided by the studies proposed above are focused on sequential learning associated with the spatial location of target objects. Therefore, changes in perceptual sensitivity and anticipatory motor planning would be related to visuospatial parameters. Amputees also demonstrate deficits in the ability to modulate the grip force of the prosthetic due to the lack of tactile feed-

back from the terminal device [159, 165]. Altering the variable which is sequentially presented provides an opportunity to examine how explicit awareness impacts perceptual sensitivity in other aspects. For example, one could be asked to transfer objects of varying mass in a sequentially predictable order, with all other visual attributes being the same. Being aware of the mass to be picked up allows for an anticipatory motor plan which prepares for the appropriate level of motor unit recruitment to control the terminal device in a more efficient manner [**Leblanc:2016**]. Having subjects transfer objects of varying deformability would also provide a means with which to introduce sequential learning. Anticipatory motor plans developed for sequential deformability would potentially include enhanced perceptual sensitivity of the muscle coordination necessary to grasp the object with the appropriate force. The use of a pre/post assessment with ADL's, as mentioned above, would allow a meaningful measure of the rehabilitative efficacy of such sequential learning.

6.9.8 Additional insight into the proposed facilitative neural network

The studies proposed above can be accomplished through behavior, kinematic and gaze behavior measures. However, as demonstrated in the presented dissertation, the neural networks involved are an important variable to consider, especially for patient populations with motor learning deficits. Having a more thorough understanding of the proposed facilitative network in healthy individuals will help in identifying the neural origin of motor learning deficits. From an analytic methodology perspective, information regarding the details of the proposed facilitative network were obtained through a correlative examination of EEG neural data. While valuable for examination of changes with the temporal precision required, the spatial resolution abilities of EEG remain a limiting factor of this neuroimaging technique. Conducting the experiments from Aims 1 and 2 incorporating a combination EEG-fMRI approach would introduce valuable additional information. An EEG-fMRI methodology would provide both the temporal resolution of EEG, with fMRI providing the spatial resolution necessary to more precisely identify the neural origins of the proposed network [316, 317]. Additionally, the correlative approach utilized provides evidence for the temporal progression of activity, but does not address potential bi-directionality of signals which may modulate

the excitability of other areas. Dynamic causal modeling has been shown to be a valuable analysis tool with which to identify, more precisely, the flow of neural signals [318, 319]. Finally, the proposed network identification and analysis was limited to specific neural regions demonstrating peak activity correlative with behavior after the first few blocks of exposure. This approach was utilized to address the proposed multiple factors proposed to impact the P3 component [198], but may have inadvertently resulted in missing neural correlates which appear even earlier for some individuals. While there is utility in examining data from an ERP perspective, the true neural origin of the positive and negative deflection remains a source of debate [178, 202]. The additional inclusion of spectral analysis would add valuable information regarding the frequency at which neural changes are associated and may reveal additional regions. Many motor learning studies have shown modulation over primary motor, premotor, medial temporal gyrus, and prefrontal areas [177, 320, 321] that were not examined in this dissertation, leaving the question of their participation in the facilitative network currently unanswered.

While current results suggest the role of a facilitative frontoparietal network mediated by visuomotor working memory capacity, it is possible that the individuals learning the sequences are just good learners. Multiple studies have demonstrated significant correlations between resting state connectivity and motor skill acquisition [140, 322]. The paradigm utilized in Aim 3 gathered resting state EEG data for subjects providing an opportunity to examination this correlation in relation to learning rates for EXP subjects and the relative connectivity measures compared to NOEXP subjects. If such a correlation exists, the next question raised would be how to improve the resting state connectivity of those individuals failing to develop awareness. As described below, examining potential methods for augmenting working memory capacity of sensorimotor information available may prove to be efficacious.

6.9.9 Exploring the role of instruction in sequential learning

From a paradigm perspective, the presented thesis utilized an incidental format with which to induce awareness. This is distinctly different from the majority of motor learning studies conducted

examining explicit awareness. As opposed to results from intentional learning paradigms, results from the current study revealed a benefit of explicit awareness on generalization for some subjects. Other subjects showed the same detrimental effect of awareness seen in intentional studies [24, 236, 59, 80, 23]. This introduces the possibility that subjects demonstrating an interference effect may have been participating in a more intentional search for a pattern from the beginning, similar to an intentional paradigm. Utilizing the proposed individualized indicator provides an opportunity to directly compare the neural progression associated with both types of awareness development to identify similarities and differences. From a rehabilitative perspective, both methods are commonly used [323, 324, 325, 326, 327]. Patients can be explicitly told the sequence of movements to execute, an intentional approach, or can be given an explicit overall goal while allowing the patient to discover the motor sequence, an incidental approach. Examining the neurobehavioral differences between the two would provide valuable additional information for guiding therapists in the identification of the most appropriate strategy for a particular patient [13, 14, 328].

6.9.10 Making the switch from incidental awareness to an intentional search

Results from Aim 2 revealed two unique responses to the introduction of a transfer sequence for subjects demonstrating awareness behavior on the priming sequence. As all subjects expressed that they were expecting a sequence in the transfer condition, it can assumed that the approach to the transfer task was different from that utilized at the beginning of the priming sequence, potentially to an intentional explicit search. As some subjects in Aim 2 successfully transferred the initially learned skill, while others did not, further exploration into the beneficial and/or detrimental interactions between the initial, incidentally developed awareness and the utilization of an intentional search strategy could provide insight the mechanisms behind these differential responses. For EXP subjects, who demonstrated a cingulo-driven strategy, the initial development of awareness may have served to enhance the perceptual sensitivity to both the visual and somatosensory cues. The utilization of this cingulo-driven maximization strategy [256] is suggested to involve a focus on the relationship between predictable stimuli rather than memorizing the exact order.

From a motor learning perspective, this may be reflective of acquiring the sequence in chunks [329], an optimization process involving cingulo-striatal connections typically noted with implicit learning [63, 66]. The additional observation of a significant decrease in PCC activation for the EXP subjects, may be reflective of fronto-parietal disengagement patterns noted to be present during consolidative and slow-learning processes [36]. Acquiring the priming sequence as chunks through cingulo-striatal connections, would present an advantage for the transfer sequence as it would potentially reduce the relative number of elements to maintain in working memory. The increased activity over the cingulate, with continued disengagement of the PCC, during the transfer sequence, for EXP subjects only, suggests that they utilized the cingulo-striatal connections of the implicit system to acquire the new transfer sequence prior to recruiting the frontoparietal networks of the explicit system. Subjects utilizing the precuneus-driven memorization strategy in the priming sequence, however, may have not fully established a chunking execution of the sequence, and therefore, been limited by working memory capacity for the transfer sequence. Studies examining intentionally developed explicit awareness have noted the early involvement of visuospatial working memory [71, 63]. The maintained fronto-parietal activation observed for EXP_NOEXP subject suggests these subjects are not moving out of the fast learning phase [36]. The enhanced precuneus and PCC neural activation patterns, along with the lack of activity suggestive of cingulo-striatal recruitment, suggest that the EXP_NOEXP subjects may have been involved in an intentional search, driven by the explicit system, for both the priming and transfer sequences. As mentioned earlier, additional studies examining the neural networks involved from an EEG-fMRI perspective, may provide additional insight into the similarities and differences between the two differential responses seen with explicitly aware primed subjects in Aim 2. Many rehabilitative approaches may initially utilize an incidental, discovery-based approach, followed by more intentionally focused, explicit instruction sessions. Therefore, understanding how to best incorporate the recruitment of the implicit and explicit systems within the context of incidental versus intentional discovery may help better guide such rehabilitative practices.

6.9.11 Addressing working memory capacity limitations

The role of working memory capacity was found to be an important factor in both Aims 2 and 3. This is in line with multiple studies which have correlated working memory capacity with motor skill acquisition [201, 158, 2]. Therefore, exploring the impact of augmenting, or disrupting, working memory would provide valuable information regarding the precise role of working memory. This could be done utilizing methods recognized to alter working memory. Multiple studies have demonstrated the efficacy of exercise for improving working memory suggesting that a bout of aerobic exercise may improve motor sequence learning [250, 264]. Studies examining pharmacological agents, such as caffeine, have also demonstrated beneficial effects on working memory [263, 245, 248]. In addition to pharmacological augmentation exploration, utilization of tCDS provides a method for examining the effects of stimulating specific brain regions [259, 260, 261, 262]. TMS studies would allow for examination of the effect of disrupting regions implicated in working memory on sequential motor learning at varying times throughout the learning progression. The current study was also restricted primarily to 7-element sequences, with longer sequences used for comparative purposes only. As previous studies have demonstrated explicit awareness occurring more frequently with shorter sequences, examining the learning progression with shorter sequences would be valuable. This information may reveal the level of generalizability of the facilitative network across all individuals, regardless of working memory capacity.

6.10 Conclusion

Results from the current dissertation provide information from an individualized perspective, revealing valuable patterns which are unique to individuals successfully acquiring motor sequence awareness. Having a method with which to explore the individualized nature of motor learning provides valuable information for rehabilitative measures to select the most appropriate intervention for a given individual's current neural state.

Appendices

APPENDIX A

EDINBURGH HANDEDNESS INVENTORY

Subject Number: _____
Date: _____
Age _____

Edinburgh Handedness Inventory

Which hand do you prefer to use when:

Task	Left	Right	No Preference
1. Writing			
2. Drawing			
3. Throwing			
4. Using scissors			
5. Brushing teeth			
6. Using a knife (without a fork)			
7. Using a spoon			
8. Using a broom (upper hand)			
9. Striking a match			
10. Opening a jar (holding the lid)			
Total			
Handedness Score			

-1.0 Pure left hander <-- **-0.5** Mixed left hander <-- **0.0** Neutral --> **+0.5** Mixed right hander --> **+1.0** Pure right hander

Handedness score is calculated using this formula: (Right - Left) / (Right + Left).

Musical & Video Garme Experience

Have you had any formal musical training?	Yes / No
If yes, how many years of formal musical training did you have?	0-1 yrs / 1-2 yrs / 2-3 yrs / 3+ yrs
What musical instrument(s) have/do you play?	
How recently have you played?	0-1 yrs / 1-3 yrs / 3-5 yrs / 5+ yrs
Do you consider yourself to be an active video game player?	Yes / No
If yes, during an average week, how many hours will you spend playing video games?	< 1 hr / 1-3 hrs / 3-5 hrs / 5-7 hrs / 7 hrs +

APPENDIX B

TRINITY AMPUTEE AND PROSTHESIS EXPERIENCE SCALE

PLEASE RESPOND TO THE QUESTIONS BELOW

FOR THE DEVICE YOU WERE USING DURING THE EXPERIMENT

ANSWER AS YOU WOULD HAVE NEAR THE TIME OF THE EXPERIMENT (XX/XX/20XX)

A) What is the power source for the device? Body-powered harness Myoelectric

B) What is the terminal device? Hook Hand

C) How many hours per day do you use your device? _____ hrs /day

	Strongly disagree	**Disagree**	**Neither agree nor disagree**	**Agree**	**Strongly agree**
1) I have adjusted to having an artificial limb……..	[1]	[2]	[3]	[4]	[5]
2) As time goes by, I accept my artificial limb more……	[1]	[2]	[3]	[4]	[5]
3) I feel that I have dealt successfully with this trauma in my life…….	[1]	[2]	[3]	[4]	[5]
4) Although I have an artificial limb, my life is full……	[1]	[2]	[3]	[4]	[5]
5) I have gotten used to wearing an artificial limb……..	[1]	[2]	[3]	[4]	[5]
6) I don't care if somebody looks at my artificial limb..	[1]	[2]	[3]	[4]	[5]
7) I find it easy to talk about my artificial limb……	[1]	[2]	[3]	[4]	[5]
8) I don't mind people asking about my artificial limb..	[1]	[2]	[3]	[4]	[5]
9) I have a difficulty in talking about my limb loss in conversation……	[5]	[4]	[3]	[2]	[1]
11) An artificial limb interferes with the <u>ability</u> to do my work……..	[5]	[4]	[3]	[2]	[1]
12) Having an artificial limb makes me more dependent on others than I would like to be……..	[5]	[4]	[3]	[2]	[1]
13) Having an artificial limb limits the <u>kind</u> of work that I can do……	[5]	[4]	[3]	[2]	[1]
14) Being an amputee means that I can't do what I want to do…….	[5]	[4]	[3]	[2]	[1]
15) Having an artificial limb limits the <u>amount</u> of work I can do……	[5]	[4]	[3]	[2]	[1]

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VITA

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